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(71) Applicant *(for all designated States except US)*: **GENFIT** [FR/FR]; Parc Eurasanté, Lille Métropole, 885, Avenue Eugène Avinée, F-59120 Loos (FR).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **HUM, Dean, W.** [CA/FR]; 26, Allée des Fenaisons, F-59910 Bondues (FR).

(74) Agents: **BECKER, Philippe** et al.; Cabinet Becker et Associés, 35, rue des Mathurins, F-75008 Paris (FR).

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(54) Title: A NOVEL ZINC FINGER PROTEIN AND USES THEREOF

(57) Abstract: The present invention relates to the identification of a novel protein, TReP-132, involved in the regulation of gene expression. This invention relates to the characterization of the function of this protein, particularly as a transcription factor involved in steroidogenesis, as well as of metabolic partners thereof. This invention also includes compositions and methods of using said protein or corresponding nucleic acids, as well as variants thereof, for screening, diagnostic and/or therapeutic purposes, more particularly for diagnosis of, therapy of, or screening compounds active in steroid hormone related disorders or in lipid disorders.

A Novel Zinc Finger Protein and uses thereof

The present invention relates to the identification of a novel protein, TReP-132, involved in the regulation of gene expression. This invention relates to the characterization of the function of this protein, particularly as a transcription factor involved in steroidogenesis, as well as of functional partners thereof. This invention also includes compositions and methods of using said protein or corresponding nucleic acids, as well as variants thereof, for screening, diagnostic and/or therapeutic purposes, more particularly for diagnosis of, therapy of, or screening compounds active in steroid hormone related disorders such as adrenal hyperplasia, adrenal hypoplasia, atypical sexual differentiation, precocious puberty, sexual dysfunction and hormone dependent cancers (e.g., breast cancer, prostate cancer, etc.), as well as in various lipid disorders, e.g., atherosclerosis, arteriosclerosis, cardiovascular diseases, obesity, diabetes, heart infarction, hypercholesterolemia, hyperlipidemia, etc.

Cytochrome P450scc is a mitochondrial enzyme that catalyzes the conversion of cholesterol to pregnenolone and is the first step in the synthesis of all steroid hormones (reviewed in (62)). The CYP11A1 gene which encodes P450scc is expressed in steroidogenic tissues, which include the adrenal, ovary, testis, placenta, and brain (58, 87). In each of these tissues, steroid hormones play important physiological roles, which include regulation of tissue development, progression of secondary sexual characteristics and maintenance of homeostasis. The hormonal regulation and developmental pattern of expression of P450scc are specific to each steroidogenic tissue, for example adrenocorticotropin increases steroidogenesis and accumulation of P450scc transcript in the human adrenal cortex, and similar effects are seen with luteinizing hormone (LH) and follicle stimulating hormone in human ovarian granulosa cells, and with LH and human chorionic gonadotropin in human testicular Leydig cells (24, 83, 99). In each case, interaction of the tropic hormone with the cell surface receptor activates G proteins leading to activation of the cAMP/PKA and Ca2+/PKC pathways which regulate P450scc gene transcription (82).

The human P450scc cDNA has been cloned and the gene mapped to chromosome 15q22.33-q23 (9, 14). Preliminary studies on the 5'-flanking region of the gene

demonstrated the ability of a 2.5 kb DNA fragment to confer basal and cAMP responsive activity when transiently transfected into mouse adrenal Y1 tumor cells (32, 33, 67). Subsequent studies in mouse Leydig MA-10 (30), I-10 (6), human placenta JEG-3 (66) and adrenal NCI-H295 (79) cells identified regions of the 5'-flanking DNA which conferred basal promoter activity and response to cAMP. However, it is apparent that different elements are utilized in a cell-type specific manner. The cAMP-responsive element identified in human JEG-3 cells is different than the element identified when studies were performed in mouse Y1, MA-10 and I-10 cells (22, 66). In these mouse cell lines, cAMP-responsive elements were localized to sites in the vicinity of nucleotides –1620 and -600, whereas in JEG-3 cells cAMP-response was conferred by a proximal region around nucleotide –117 (22, 97); however, neither of these elements were functional in NCI-H295 cells (79). Positive *cis*-acting elements have also been identified, which confer expression of P450scc gene promoter activity in steroidogenic cell lines, but not in nonsteroidogenic cells. However, it is also apparent that some elements, such as those found between nucleotides –1931 and –1822, can increase promoter activity in Y1 and MA-10 cells, but not in NCI-H295 and JEG-3 cells (8). In other studies, the region between –155 and –131 was found to significantly increase promoter activity in JEG-3 cells (28, 66).

These putative *cis*-acting elements in the 5'-flanking region of the human P450scc gene have been demonstrated to interact with multiple proteins, which include CREB/ATF (97), NF1- and Sp1-like proteins, and in particular the steroidogenic factor-1 (SF-1). The orphan nuclear receptor SF-1 also known as adrenal binding protein (Ad4BP) has been demonstrated to promote cell specific expression of the human P450scc gene promoter in Y1 cells (69, 89, 97). The human P450scc gene promoter is inactive in nonsteroidogenic CV-1 cells, but could be activated by expression of exogenous SF-1 (26, 69). In addition to the interaction of the P450scc gene 5'-flanking DNA with known proteins, the putative *cis*-acting elements have also been found to interact with unidentified presumably novel transcription factors. The positive element between –155 and –131 (-155/-131) was shown to interact specifically with at least two different proteins; however, prior to the present study the identity and role of neither of these proteins were known. Based on the many previous studies on P450scc gene expression, which demonstrate the cell type specific usage of *cis*-acting elements and involvement of

multiple *trans*-acting factors, it is obvious that a clear understanding of the mechanisms involved in the regulation of expression of this important gene will require identification and characterization of the transcription factors involved. Recently, Huang *et al.* (27) identified two proteins LBP-1b and LBP-9, which interact with the –155/-131 element and regulate reporter activity via this element.

The present invention now discloses the cloning and functional characterization of a novel protein, termed TReP-132. TReP-132 was cloned by screening a placental cDNA expression library with a region of the P450scc gene 5'-flanking region from nucleotides –155 to –131 (-155/-131). Analysis of the predicted primary structure reveals a protein of 1200 residues, which contains three zinc fingers of the C_2H_2 subtype. TReP-132 also has other motifs characteristic of transcription factors including regions rich in glutamine, proline and acidic residues. The TReP-132 primary structure contains two LXXLL motifs, which suggests the interaction of the protein with nuclear receptors such as SF-1 to regulate gene expression. The encoded protein with an apparent molecular mass of 132 kDa was shown to interact with the –155/-131 element by electrophoretic mobility shift assay (EMSA). Transient expression of this protein increased expression of a reporter construct controlled by the 5'-flanking region of the P450scc gene from nucleotides –1676 to +49. As well, the –155/-131 element was found to confer response to TReP-132. The interaction of TReP-132 with DNA, its subcellular localization in the cell nucleus and its ability to increase promoter activity indicate its function as a transcription factor. To further understand the mechanism by which TReP-132 regulates gene expression, it was shown to interact with the coregulator protein CBP/p300 to synergistically increase promoter activity. Transcripts encoding TReP-132 are expressed predominantly in the adrenal gland and testis, as well as steroidogenic cell lines, which is consistent with the potential role of this factor to regulate steroid synthesis. TReP-132 transcripts are also expressed in several other tissues examined, including the brain, thymus, stomach and heart, which indicates additional roles for TReP-132 other than regulation of steroid synthesis.

The present invention thus results from cloning and characterization of a novel protein, involved in the regulation of gene expression, particularly during steroidogenesis and lipid metabolism. The invention demonstrates that the protein regulates expression of

cytochrome P450scc, and interacts with functional partners, particularly CBP/p300 and SF1. The present invention now proposes to use the protein as a target to screen active compounds and or to identify further metabolic pathways and partners involved in steroidogenesis. The invention also relates to methods of treating steroid related diseases and lipid disorders using said protein, corresponding nucleic acids, as well as active compounds.

An object of the present invention thus resides in the use of a TReP-132 polypeptide or a nucleic acid molecule encoding the same, for the in vitro selection of compounds that regulate gene expression.

A further object of this invention resides in the use of a TReP-132 polypeptide or a nucleic acid molecule encoding the same, for the manufacture of a composition suitable to regulate gene expression in vivo.

An other object of the present invention resides in a method of regulating gene expression in vivo comprising administering to a subject a TReP-132 polypeptide or a nucleic acid molecule encoding the same.

An other aspect of this invention is the use of a compound that modulates (e.g., stimulates or represses) expression or activity of a TReP-132 polypeptide, in the manufacture of a medicament for treating steroid related disorders and/or lipid disorders, particularly cardiovascular diseases and obesity.

Still a further aspect of this invention is a method of treating steroid related disorders and/or lipid disorders, particularly cardiovascular diseases and obesity, comprising administering to a subject a compound that modulates, preferably stimulates, expression or activity of a TReP-132 polypeptide.

Further objects of this invention include a TReP-132 polypeptide, synthetic variants thereof, corresponding nucleic acids, vectors, recombinant host cells, and method of screening active compounds.

Within the context of the present invention, a TReP-132 protein or polypeptide designates a polypeptide comprising the amino acid sequence SEQ ID NO:2, as well as any variant thereof, including any naturally-occurring derivatives thereof or homologues isolated from various mammalian species. Variants more generally encompass any

polypeptide having one or several amino acid modifications as compared to the sequence of SEQ ID NO:2, including mutation(s), deletion(s), insertion(s), substitution(s), alone or in various combinations(s). Variants specifically include fragments as well as alternative splicing forms of TReP-132. More particularly, variants are any polypeptide having the property to bind CBP/p300 and/or SF-1 and/or SEQ ID NO: 3 or 4, said polypeptide being encoded by a nucleic acid sequence hybridizing, under conventional, moderate stringency, with the nucleic acid sequence of SEQ ID NO: 1 or a fragment thereof. Hybridization conditions are, for instance incubation at 42°C for 12 hours in 50% formamide, 5 X SSPE, 5 X Denhardt's solution, 0.1% SDS, 100µg/ml salmon sperm DNA and DNA probe at $1x10^6$ cpm/ml (1 X SSPE is composed of 0.15M NaCl, 10 mM NaH_2PO_4, 1.3 mM EDTA, pH 7.4). The sequence of specific examples of naturally-occurring variants is available under Genbank accession number AJ277276 or AJ277275.

Particular fragments include polypeptides having residues 1-161 or 546-628 of SEQ ID NO: 2, or sub-fragments thereof. Other fragments include portions of a TReP-132 polypeptide that bind to a CBP/p300 molecule and/or to a SF1 molecule and/or to SEQ ID NO:3 or 4. Such fragments include for instance residues 439-1200 of SEQ ID NO:2.

Still preferably, a TReP-132 protein of this invention has at least 80% identity with the amino acid sequence of SEQ ID NO: 2, more preferably at least 85%, still more preferably at least 90%, even more preferably at least 95%. Identity can be determined using known methods and commercial computer programs, such as CLUSTAL or BLAST (NCBI). Various search or alignment parameters may also be used. In a preferred embodiment, percentage amino acid (or nucleic acid) identity is determined using the CLUSTAL-W program (Compugen).

In this regard, a specific, preferred object of this invention resides in a polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

Another specific, preferred object of this invention resides in a polypeptide comprising amino acid sequence from residues 546 to 628 of SEQ ID NO: 2, or a fragment thereof comprising at least 4 consecutive residues thereof, preferably at least 5 consecutive residues thereof, even more preferably at least 6 consecutive residues thereof. More preferred fragments comprise typically between 5 and 50 consecutive amino acids, even more preferably between 6 and 40.

Another specific, preferred object of this invention resides in a polypeptide comprising amino acid sequence from residues 1 to 161 of SEQ ID NO: 2, or a fragment thereof comprising at least 4 consecutive residues thereof, preferably at least 5 consecutive residues thereof, even more preferably at least 6 consecutive residues thereof. More preferred fragments comprise typically between 5 and 50 consecutive amino acids, even more preferably between 6 and 40.

Another specific, preferred object of this invention resides in a TReP-132 polypeptide that lacks a functional LRQLL region. More specifically, the LRQLL region is located at residues 181-185 of SEQ ID NO:2. Polypeptides lacking a functional or lacking a native LRQLL region may comprise a deletion of one or more of said amino acids, or, preferably, a substitution thereof. For instance, particular polypeptides comprise a LRQLL region wherein the leucine residues were changed in alanine residues. Other polypeptides lacking such a functional region include polypeptides comprising a deletion of all or part of the amino-terminal region comprised between residues 1-185.

Another specific, preferred object of this invention resides in a polypeptide fragment of a TReP-132 polypeptide, said polypeptide fragment comprising the LRQLL region. The polypeptide fragment typically contains less than about 200 amino acids of a TReP-132 polypeptide, particularly of SEQ ID NO: 2, and comprises at least residues 181-185 thereof, preferably at least residues 178-188 thereof. A typical example is a polypeptide consisting of residues 1-200 or 1-188 of SEQ ID NO:2. Other examples are any polypeptide fragments having the LRQLL motif and containing less than about 80 amino acids, or less than about 60 amino acids. Preferably, the polypeptide fragment comprises more than 10 amino acids.

Another specific, preferred object of this invention resides in a TReP-132 polypeptide that lacks a functional Zinc Finger motif. Zinc Fingers motif comprise C2H2 motifs, as illustrated on Figure 4, and are located at residues 514-534, 1015-1037 and 1088-1108 of SEQ ID NO:2. A particular example of such a polypeptide is a TReP-132 polypeptide lacking all or part of the C-terminal region comprised between residues 1015-1200.

Another specific, preferred object of this invention resides in a TReP-132 polypeptide that lacks a functional LEMLL motif. The LEMLL motif is located at residues 863-867 of SEQ ID NO:2. A particular example of such a polypeptide is a TReP-

132 polypeptide lacking all or part of the C-terminal region comprised between residues 863-1200.

Another polypeptide of this invention is a polypeptide fragment of a TReP-132 polypeptide comprising the LEMLL motif. The polypeptide fragment typically contains less than about 100 amino acids of a TReP-132 polypeptide, particularly of SEQ ID NO: 2, and comprises at least residues 863-867 thereof, more preferably at least residues 859-870 thereof. Other examples of polypeptide fragments contain less than about 80 amino acids, or less than about 60 amino acids. Preferably, the polypeptide fragment comprises more than 10 amino acids.

Another specific, preferred object of this invention resides in a TReP-132 polypeptide that lacks a glutamine rich region located at residues 252-343 of SEQ ID NO:2, or that lacks a proline rich region located at residues 553-570 of SEQ ID NO:2, or that lacks a glutamate/proline rich region located at residues 956-1009 of SEQ ID NO:2.

It should be understood that the above modifications may be combined together. Particular examples of such polypeptides are shown on Figure 11A.

A TReP-132 polypeptide of this invention may be an isolated, purified preparation, essentially free of other naturally-occurring proteins with which TReP-132 naturally interacts. The polypeptide may be isolated from biological samples or, more preferably, a recombinant TReP-132 polypeptide, i.e., produced by expression, in any suitable host cell, of a nucleic acid molecule encoding such a polypeptide. Suitable host cells include for instance mammalian cells, prokaryotic cells or yeast cells. A preferred TReP-132 polypeptide for use in the present invention is a human TReP-132 polypeptide, even more preferably a recombinant human TReP-132 polypeptide. For use in the present invention, although essentially pure TReP-132 polypeptide preparations are preferred, it should be understood that any TReP-132 polypeptide preparation may be used, in combination with other factors, diluents, adjuvants, biological fluids, etc.

Within the context of this invention, a TReP-132 nucleic acid molecule designates any nucleic acid molecule encoding a polypeptide as defined above. The nucleic acid may be DNA or RNA, particularly genomic DNA (gDNA), complementary DNA (cDNA), synthetic or semi-synthetic DNA, messenger RNA (mRNA), etc. Typically, the nucleic acid is a cDNA. The nucleic acid sequence may be altered to improve codon usage,

eliminate secondary structures, create restriction sites, etc. In a preferred embodiment, the nucleic acid molecule comprises all or part of SEQ ID NO: 1 or a variant thereof. The term variant is as defined above. Typically, variants include nucleic acid sequence hybridizing, under conventional, moderate stringency, with the nucleic acid sequence of SEQ ID NO: 1 or a fragment thereof. Hybridization conditions are, for instance, incubation at 42°C for 12 hours in 50% formamide, 5 X SSPE, 5 X Denhardt's solution, 0.1% SDS, 100µg/ml salmon sperm DNA and DNA probe at $1x10^6$ cpm/ml (1 X SSPE is composed of 0.15M NaCl, 10 mM NaH_2PO_4, 1.3 mM EDTA, pH 7.4).

In this regard, a specific, preferred object of this invention resides in a nucleic acid molecule encoding a polypeptide of SEQ ID NO: 2, more preferably a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1.

Another specific, preferred object of this invention resides in a nucleic acid molecule comprising the nucleotide sequence encoding residues 546 to 628 of SEQ ID NO: 2, or a fragment thereof comprising at least 4 consecutive residues thereof, preferably at least 5 consecutive residues thereof, even more preferably at least 6 consecutive residues thereof. More preferred fragments comprise typically between 5 and 50 consecutive amino acids, even more preferably between 6 and 40.

Another specific, preferred object of this invention resides in a nucleic acid molecule comprising the nucleotide sequence encoding residues 1 to 161 of SEQ ID NO: 2, or a fragment thereof comprising at least 4 consecutive residues thereof, preferably at least 5 consecutive residues thereof, even more preferably at least 6 consecutive residues thereof. More preferred fragments comprise typically between 5 and 50 consecutive amino acids, even more preferably between 6 and 40.

Further specific objects of this invention are nucleic acid molecules encoding polypeptides as defined above, i.e., TReP-132 polypeptides lacking a LRQLL region and/or a LEMLL region and/or a Zinc Finger motif and/or a glutamine rich domain, as well as TReP-132 polypeptide fragments comprising a LRQLL region or a LEMLL region or a Zinc Finger motif.

Recombinant expression vectors comprising a nucleic acid as defined represent further objects of the present invention. These vectors can be used to express a TReP-132

polypeptide of this invention in vitro, ex vivo or in vivo, to create transgenic or "Knock Out" non-human animals, to amplify the nucleic acids, to express antisense RNAs, etc.

The vectors usually comprise the above nucleic acid operably linked to regulatory sequences, e.g., a promoter, a polyA, etc. The term "operably linked " indicates that the nucleic acid and regulatory sequences are functionally associated. The vectors may further comprise one or several origin of replication and/or selectable markers. The promoter region may be homologous or heterologous with respect to the nucleic acid, and provide for ubiquitous, constitutive, regulated and/or tissue specific expression, in any appropriate host cell, including for in vivo use. Examples of promoters include bacterial promoters (LacZ, T7, lambda PR, trp promoter, etc.), viral promoters (LTR, TK, SV40, CMV-IE, etc.), mammalian gene promoters (apolipoprotein, albumin, PGK, etc), and the like.

The vector may be a plasmid, cosmid, phage, virus, etc. Plasmid vectors may be prepared from commercially available vectors such as pBluescript, pUC, pBR, etc. Viral vectors may be produced from baculoviruses, retroviruses, adenoviruses, AAVs, etc., according to recombinant DNA techniques known in the art (see for instance WO93/25234, WO 94/06920, etc.).

In this regard, a particular object of this invention resides in a recombinant virus encoding a TReP-132 polypeptide as defined above. The recombinant virus is preferably replication-defective, even more preferably selected from E1- and/or E4-defective adenoviruses, Gag-, pol- and/or env-defective retroviruses and Rep- and/or Cap-defective AAVs.

A further object of the present invention resides in a recombinant host cell comprising a nucleic acid or a vector as defined above. Typical host cells are prokaryotic (e.g., bacteria) or eukaryotic (e.g., yeast, mammalian, insect, etc.) host cells. Specific examples include E.coli, Kluyveromyces yeasts, Saccharomyces, established mammalian cell lines (e.g., Vero, CHO, 3T3, HeLa, NCI-H295, JEG-3, etc.) as well as primary or established mammalian cell cultures.

The present invention also relates to a method for producing a TReP-132 polypeptide, said method comprising culturing in vitro or ex vivo a recombinant host cell as described above and collecting the polypeptide.

A further object of this invention resides in an antibody that binds a TReP-132 polypeptide as defined above, as well as fragments or derivatives of said antibody having substantially the same antigenic specificity. The antibody may be a polyclonal or a monoclonal. Derivatives include fragments (e.g., Fab, Fab'2, CDRs, etc) as well as humanized antibodies, poly-functional antibodies, Single Chain antibodies (ScFv), etc. These may be produced according to conventional methods, including immunization of an animal and collection of serum (polyclonal) or spleen cells (to produce hybridomas by fusion with appropriate cell lines). Preferred antibodies of this invention are prepared by immunization with a fragment of a TReP-132 polypeptide comprising at least residues 1-161 or 546-628 of SEQ ID NO: 2, or other polypeptides as defined above, or an immunogenic sub-fragment thereof, e.g., a subfragment comprising at least an epitope, preferably of at least 5 amino acids. A particular antibody is a polyclonal antibody prepared by immunization with a full-length TReP-132 polypeptide.

Particularly preferred antibodies are those who bind specifically to a TReP-132 polypeptide, i.e., that substantially do not bind other molecules with a high affinity, more preferably that have an affinity for a TReP-132 polypeptide that is at least 5 times higher than for another naturally-occurring protein.

As indicated, the invention stems from the identification and characterization of a novel protein involved in gene regulation, particularly in the synthesis of steroid hormones and/or the metabolism of lipids, including. The invention thus proposes novel methods of screening active compounds using a TReP-132 polypeptide or nucleic acid as a target.

Within the context of this invention, "active compounds" designates any compound having the ability to modulate (or regulate) the expression and/or activity of a TReP-132 polypeptide. In view of the present newly discovered properties of TReP-132, said active compounds exhibit a high potential lipid metabolism or steroid hormone synthesis regulating activity. More preferred active compounds are suitable for treating steroid hormone related diseases such as adrenal hyperplasia, adrenal hypoplasia, atypical sexual differentiation, precocious puberty, sexual dysfunction and hormone-dependent cancers (e.g., breast cancer, prostate cancer, etc.), cardiovascular diseases,

hyperlipidaemia, hypercholesterolemia, obesity, hormonal dysfunctions, atherosclerosis, arteriosclerosis, diabetes, etc. Active compounds may be any synthetic compound, including organic products, lipids, peptides, nucleic acids (e.g., antisense), etc. Compounds that modulate TReP-132 expression include compounds that modulate TReP-132 transcription, splicing, translation and/or post-translational modification. Compounds that modulate TReP-132 activity include compounds that inactivate TReP-132, activate TReP-132, interfere with the binding of TReP-132 to other partners or nucleic acids, etc. Preferred compounds are able to modulate the binding of TReP-132 to receptors, such as nuclear receptors, co-factors or nucleic acid binding sites. Such receptors include, for instance, all or part of CBP/p300, SF-1 or SEQ ID NO: 3 or 4. Most preferred active compounds are able to stimulate or reproduce TReP-132 activity and/or to exert a agonist effect thereof.

This invention thus proposes new screening methods to identify, select, improve or characterize active compounds. The screening methods include binding assays, transcriptional assays, functional assays, etc., in vitro or using cell or membrane extracts or using cell systems.

In this regard, a particular object of this invention lies in a method of screening, selecting or identifying compounds that regulate gene expression, the method comprising contacting in vitro a candidate compound with a TReP-132 polypeptide or a fragment thereof, and selecting the candidate compound that binds said TReP-132 polypeptide or fragment thereof, the binding to a TReP-132 polypeptide or a fragment thereof being indicative of a gene expression regulation activity.

An other object of this invention resides in a method of screening, selecting or identifying compounds that regulate gene expression, the method comprising contacting in vitro a candidate compound with a TReP-132 receptor in the presence of a TReP-132 polypeptide or a fragment thereof, and selecting the candidate compound(s) that modulate(s) the binding of said TReP-132 polypeptide or fragment thereof to said receptor, said modulation being indicative of a gene expression regulation activity. Binding may be assayed in the presence of a TReP-132 polypeptide.

The TReP-132 receptor may be any nuclear receptor, co-factor, binding partner or nucleic acid molecule to which TReP-132 binds or with which TReP-132 interacts, or a part thereof comprising the binding site.

As an example, the receptor may be a nucleic acid molecule comprising the sequence SEQ ID NO: 3 or 4 or a functional variant thereof. SEQ ID NO: 3 and 4 represent a cis-acting region in the CYP11A1 gene promoter that is regulated by TReP-132. Any functional variant of this sequence may be used as well, i.e., any nucleic acid sequence having one or several modifications as compared to SEQ ID NO: 3 or 4 and retaining the ability to bind a TReP-132 polypeptide.

As another example, the receptor may be all or part of a CBP/p300 or of a SF-1 polypeptide. Indeed, the present invention shows that TReP-132 interacts with CBP/p300 and/or SF-1 and that said interaction is likely to contribute to the regulatory activity of TReP-132, especially in the regulation of genes involved in steroidogenesis. Fragments of CBP/p300 or SF-1 may be used as well, such as any fragment that retains the domain involved in the interaction with TReP-132.

A particular receptor is a polypeptide comprising at least amino acid residues 1-451, 1460-1891 or 1892-2441 of CBP/p300. As illustrated in the examples, these regions are responsible for binding to TReP-132.

Another particular receptor is a polypeptide comprising all or part of SF-1. SF-1 (also called AD4BP for adrenal 4-binding protein) is a key transcription factor that controls P450scc gene expression. Homologue to the *Drosophilia* nuclear receptor fushi tarazu factor 1, SF-1 is an orphan nuclear receptor that binds to the PyCAAGGTCA sequence. It was initially isolated as a major regulator of the cytochrome P450 steroid hydroxylases in adrenocortical and gonadal cells (Rice, 1991; Lala et al., 1992; Morohashi et al., 1992, Morohashi et al., 1995). In particular, SF-1 binds and activates P450scc 5'-flanking DNA regions. The sequence of SF-1 is available at Genbak accession number AB000490. The amino acid sequence is represented below as SEQ ID NO: 6:

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MDYSYDEDLDELCPVCGDKVSGYHYGLLTCESCKGFFKRTVQNNKHYTCT    50
ESQSCKIDKTQRKRCPFCRFQKCLTVGMRLEAVRADRMRGGRNKFGPMYK   100
RDRALKQQKKAQIRANGFKLETGPPMGVPPPPPPPPDYMLPPSLHAPEPK   150
ALVSGPPSGPLGDIGAPSLPMSVPGPHGPLAGYLYPAFSNRTIKSEYPEP   200
YASPPQQPGPPYSYPEPFSGGPNVPELILQLLQLEPEEDQVRARIVGCLQ   250
EPAKSGSDQPAPFSLLCRMADQTFISIVDWARRCMVFKELEVADQMTLLQ   300
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NCWSELLVLDHIYRQVQYGKEDSILLVSGQEVELSTVAVEAGSLLHSLVL 350
RAQELVLQLHALQLDRQEFVCLKFLILFSLDVKFLNNHSLVKDAQEKANA 400
ALLDYTLCHYPHCGDKFQQLLLCLVEVRALSMQAKEQYLYHKHLGNEMPR 450
NNLLIEMLQAKQT 463

The present invention now demonstrates that TReP-132 and SF-1 interact and synergize to activate the P450scc promoter. Mutation at the -46 SF-1 binding site abolished P450scc activity conferred by co-transfections of these proteins. Moreover, at limiting concentrations of TReP-132, SF-1 and TReP-132 interact to activate P450scc promoter in synergy with p300. The domains involved in interaction between TReP-132 and SF-1 was shown to be the LRQLL motif of TReP-132, found at position 181-185 of TReP-132, and both the AF-2 hexamer (LLIEML) and sequence at position 118 to 187 of SF-1 (SEQ ID NO: 6).

In a particular embodiment, the TReP-132 receptor thus comprises all or part of SF-1 polypeptide, more specifically at least amino acid residues 106-187 or 118-187 of SF-1, or at least the LLIEML sequence. This invention further demonstrates that the region of TReP-132 responsible for the interaction with SF-1 lies within the putative NR boxes comprising the LRQLL and/or the LEMLL domains. A particular embodiment of this invention thus resides in a method of selecting active compounds, the method comprising screening test compounds for their ability to interfere with the interaction between a TReP-132 polypeptide or a fragment thereof comprising at least the LRQLL and/or the LEMLL domain, and the SF-1 polypeptide or a fragment thereof comprising at least amino acid residues 106-187 or 118-187 of SF-1, or at least the LLIEML sequence. As indicated above, the assay can be performed in vitro (e.g., double hybrid system or FRET analysis), or in a cellular system, in the presence of a reporter construct.

Other TReP-132 receptors (e.g., binding partners and/or co-factors) may be isolated using various techniques, and used in the above screening methods. In particular, considering that TReP-132 functions as a coregulator of the nuclear receptor SF-1 and that multiple potential interaction domains are found in TReP-132, this molecule can be used to identify other nuclear receptor partners or other partners involved in gene transcription. In a two hybrid system, TReP-132 can be fused with the DNA-binding domain of the yeast GAL4 protein to form a chimeric protein that interacts with the

GAL4 binding site in a reporter plasmid. When expressed in a cell, these two entities can be used to screen a cDNA library which expresses proteins fused to the activation domain of the VP16 protein. The identification of proteins which interact with TReP-132 to regulate transcription would identify potential therapeutic target genes that can be regulated by TReP-132.

If there are known proteins (nuclear receptors and other transcription factors) suspected to interact with TReP-132, pull-down analysis, coimmunoprecipitation/Western blot and two-hybrid analysis can be performed. In all these approaches the concept is to use TReP-132 as a bait to assess the interaction with the other protein. In a pull-down analysis, a GST-TReP chimera can be immobilized on a matrix and interacted with a labelled (radioactive) protein. The retention of the labelled protein would be indicative of interaction. The reverse experiment can also be done with labelled TReP-132 and the other protein expressed as a GST chimera. The coimmunoprecipitation/Western blot approach would be to transfect TreP-132 and the other protein into a cell, and then do an immunoprecipitation specific for one of the proteins, and then do a Western blot specific for the other protein, which would assess if there is interaction between the two proteins in the cell. These physical interaction approaches may be coupled with functional interaction studies. TreP-132 and the other partner can be co expressed with a reporter gene driven by the gene promoter of interest. The expected result would be for the coexpression of TreP-132 and its partner to regulate the reporter gene.

In the screening assay according to the present invention, the contacting may be performed in vitro, in a cellular or acellular assay. For instance, the contacting may be performed using isolated polypeptides and receptors. The contacting may also be performed in a cell system, wherein the cell expresses the TReP-132 polypeptide and the receptor, and is contacted with the test compound.

In this regard, a further embodiment of this invention is a method of screening, selecting or identifying compounds that regulate expression, particularly expression of a gene involved in steroid synthesis, the method comprising contacting a candidate compound with a cell comprising a reporter construct, said reporter construct comprising a reporter gene under the control of a promoter comprising SEQ ID NO: 3 or 4 or a functional variant thereof, and selecting the candidate compound that modulates (e.g.

stimulates or reduces) expression of the reporter gene, said modulation being indicative of a gene expression regulation activity.

In a preferred embodiment, the assay comprises contacting a candidate compound with a cell comprising a reporter construct, said reporter construct comprising a reporter gene under the control of a promoter comprising SEQ ID NO: 3 or 4 or a functional variant thereof, the contacting being performed in the presence of a TReP-132 polypeptide, and selecting the candidate compound(s) that modulate expression of the reporter gene, said modulation being indicative of a gene expression regulation activity.

In an other embodiment, this invention relates to a method of screening, selecting or identifying compounds that regulate expression, particularly expression of a gene involved in steroid synthesis, the method comprising contacting a candidate compound, in the presence of a TReP-132 polypeptide, with a cell comprising a reporter construct, said reporter construct comprising a reporter gene under the control of an SF-1-responsive promoter, and selecting the candidate compound that modulates (e.g. stimulates or reduces) expression of the reporter gene, said modulation being indicative of a gene expression regulation activity.

Most preferably, the SF-1-responsive promoter is a promoter comprising one or several copies of SEQ ID NO: 5 (TCAAGGCCA) or a complementary sequence or a functional variant thereof. Such a promoter is, for instance the promoter of the cytochrome P450 gene or a fragment thereof comprising at least the nucleotide residues –46 to –38 thereof. The cell should contain or express a SF-1 polypeptide.

Even more preferably, the assay comprises measuring the activity of the reporter gene in the absence of a test compound, and comparing said activity with the activity obtained in the presence of the test compound.

Most preferably, the cell assays as described above use a cell that contains a reporter system as described above and that further encodes a TReP-132 polypeptide. In a further preferred embodiment, the cell also expresses SF-1 polypeptide. Particular cells include stable cells that constitutively express a TReP-132 polypeptide , as well as cells providing for regulated expression. In this regard, expression of the TReP-132 polypeptide can be accomplished using a promoter as described above (e.g., a CMV

promoter), or a regulated promoter (e.g., tetracycline-regulated promoter for instance). Stable cells preferably contain a TReP-132 polypeptide expression cassette integrated in their genome. The cells may be any recombinant host cell as described above. The reporter gene may be any gene whose transcription or translation can be detected, including marker genes that produce light, color or that cause or compensate a deficiency in a cell. Such reporter genes are preferably selected from galactosidase, lactamase, luciferase, GFP, etc.

In the reporter construct, a promoter comprising SEQ ID NO: 3 or 4 and/or 5 or a functional variant thereof is typically being used. Indeed, as disclosed in the examples, the presence of such a region confers TReP-132-sensitivity to a promoter region. The promoter may be, for instance, a promoter that naturally comprises such a sequence (e.g., the CYP11A1 gene promoter). The promoter may be any artificial promoter comprising such a sequence, in one or several copies, typically in one to 10 copies, preferably between 1 and 5 copies. The promoter may thus comprise all or a transcriptionally functional part of a promoter selected from viral promoters and mammalian promoters, fused to one or several copies of SEQ ID NO: 3 or 4 and/or 5 or a functional variant thereof. Examples of such promoters are disclosed in the experimental section.

The screening assays allow the identification of compounds that activate, repress, reproduce or mimic TReP-132. The above screening assays are particularly suited for screening, selecting or identifying compounds that regulate expression of genes involved in steroidogenesis and lipid metabolism.

As indicated, a preferred screening method comprises contacting a test compound with a cell expressing a reporter gene under the control of a promoter comprising SEQ ID NO: 3 or 4 or 5 or a functional variant thereof, and determining the ability of the said test compound to modulate reporter expression.

These screening assays may be combined with secondary assays to validate the functional and therapeutic properties of the compounds.

The assay may be performed in any suitable device, such as plates, tubes, dishes, flasks, etc. Typically, the assay is performed in multi-well plates. Several test compounds can be assayed in parallel. Furthermore, the test compound may be of various origin,

nature and composition. It may be any organic or inorganic substance, isolated or in mixture with other substances. The compounds may be all or part of a combinatorial library of products, for instance.

The selected compounds have the ability to regulate gene expression, particularly to stimulate or inhibit the expression of genes involved in steroidogenesis and lipid metabolism. The compounds represent high potential drug candidates for treatment of various pathological conditions associated with steroid hormone related disorders such as adrenal hyperplasia, adrenal hypoplasia, atypical sexual differentiation, precocious puberty, sexual dysfunction and hormone cancers (e.g., breast cancer, prostate cancer, etc.) or with lipid metabolism disorder, such as cardiovascular diseases, obesity, etc.

In this regard, the invention also relates to pharmaceutical compositions comprising a TReP-132 polypeptide or a nucleic acid molecule encoding the same or a compound that modulates expression or activity of a TReP-132 polypeptide. The composition may comprise any conventional excipient or vehicle, such as saline solution, isotonic buffer, stabilizing agents, emulsifying agents, etc. Preferred compounds are those selected, identified, characterized and/or improves using an assay as described above.

The present invention also relates to compositions, kits and methods of detecting, diagnosing or monitoring the presence of or predisposition to or evolution of a steroid hormone related disease or a lipid metabolism disorder. The method comprises determining in vitro the status of the TReP-132 gene or polypeptide in a subject. Determining the status of the TReP-132 gene or polypeptide comprises detecting the presence of variant(s), polymorphism(s) or genetic alteration(s) in the TReP-132 gene or polypeptide in said subject. The alteration in the TReP-132 gene or polypeptide may be any genetic alteration, such as a mutation, a deletion, an inversion, an addition and/or a substitution of one or more residues in said gene or encoded polypeptide. Preferred genetic alterations are mutations in a coding or non-coding region of the TReP-132 gene, including exons, introns and regulatory sequences (such as 5'- and 3'-untranslated sequences, promoters, etc.), particularly mutations in a coding region leading to a change

in amino acid sequence in the encoded polypeptide, e.g., an amino acid substitution, a frameshift and/or a truncated polypeptide sequence.

Typically, the method is performed on a biological sample from a subject (e.g., a blood sample, urine sample, serum, biopsy, etc.) and comprises a determination of the presence of an alteration in the sequence of the TReP-132 gene or mRNA or polypeptide. Determination of such an alteration (e.g., mutation, substitution, deletion, splicing, etc.) can be carried out using various techniques known in the art such as by (partial) sequencing, chromatography, electrophoresis, gel migration, hybridization, etc. Other nucleic acid detection methods can be employed, such as LCR (Ligase Chain Reaction), TMA (Transcription Mediated Amplification), PCE (an enzyme amplified immunoassay) and bDNA (branched DNA signal amplification) assays.

The detection or quantification method usually comprises a treatment of the sample to render the gene or polypeptide available for detection. Treatment may comprise any conventional fixation techniques, cell lysis (mechanical or chemical or physical), or any other conventional method used in immunohistology or biology, for instance.

In a first variant, the present invention, provides a method of detecting the presence of an altered TReP-132 polypeptide. The presence of a TReP-132 polypeptide can be determined by any suitable technique known in the art, such as by immuno-assay (ELISA, EIA, RIA, etc.). This can be made using any affinity reagent specific for a TReP-132 polypeptide, more preferably any antibody or fragment or derivative thereof. The antibody (or affinity reagent) may be labelled by any suitable method (radioactivity, fluorescence, enzymatic, chemical, etc.).

In an other variant, the method comprises determining the presence of an altered TReP-132 gene in the sample, as an indication of the presence of or risk of developing a steroid-hormone related or lipid metabolism related disease. Preferably, the method comprises determining the presence of polymorphisms or mutations in the TReP-132 gene. This may be done by prior amplification of all or part of the TReP-132 gene using specific primers, and detection of the presence of mutation(s) by (partial) sequencing or hybridization with specific probes, for instance. In this regard, the invention encompasses methods of detecting the presence of an alteration in the TReP-132 gene in a biological

sample from a subject. The invention also encompasses primers that are complementary to and specific for the TReP-132 gene or RNA and allow the amplification of all or a portion of the TReP-132 gene or RNA in a sample. Said primers contain, preferably, less than about 50 nucleotides, and are complementary to a region of the TReP-132 gene or RNA. The invention also includes compositions or kits comprising a pair of primers as discussed above.

Other aspects and advantages of the present invention will be disclosed in the following examples, which should be regarded as illustrative and not limiting the scope of this application.

LEGEND TO THE FIGURES

Fig. 1. Three copies of the –155/-131 element from the P450scc gene 5'-flanking region were fused upstream of the TK32 reporter plasmid, and the construct was transiently transfected into human HK293, HeLa, NCI-H295 and JEG-3 cells. This element confers promoter activity in JEG-3 and NCI-H295 cells, but not in HK293 and HeLa cells.

Fig. 2. (A) Schematic of a subset of the cDNA clones isolated. P1, the initial partial cDNA purified, was used as a probe to isolate the subsequent clones. The clones represented by a bar are denoted by names on the left. The shaded area on the right represents the portion of the 3'-untranslated region which is found only in the longer transcript. The regions denoted I and II were amplified by PCR using oligonucleotides denoted by the arrows. The resulting amplified cDNA products were radiolabeled and used as probes in Northern blot analysis. The shaded region denoted by III was amplified by PCR to assess the size of the 3'-untranslated region in clone P1-CLF and was found to be approximately 2 kb in length. The Eco RI site used to construct the full length TReP-132 cDNA from clones P1-CL4 and P1-CL6 is denoted by the vertical arrows. The initiation codon (ATG) and the stop codon (TAA) is denoted and the location of the C_2H_2

zinc finger motifs are represented by the closed black boxes. The length of the cDNAs represented by the open bars are drawn to scale.

(B) Northern blot analysis of TReP-132 transcripts. 2 μg of JEG-3 cell mRNA was separated on a 1.1% agarose formaldehyde gel and the blot was hybridized with a 5' probe and a 3' probe, depicted in panel A as regions I and II respectively.

Fig. 3. (A) Northern blot analysis of mRNA from human tissues. Each lane contains 2 μg of mRNA and the blot was hybridized with a radiolabelled probe which contains nucleotides 532 to 1035 of TReP-132. The blot on the left was exposed for 48 hours and the blot on the right for 96 hours to X-omat film at -70 C. The panels on the bottom show the results of hybridization of the same blots with a β-actin probe.

(B) RNase protection analysis of TReP-132 transcripts in cell lines. 20 μg of total RNA from the different cell lines were hybridized with a radiolabeled TReP-132 riboprobe in combination with a 18S riboprobe for normalization. Following digestion with RNase, samples were separated on a urea 4% polyacrylamide gel to resolve the protected fragments. Transcript expression was detected in all the steroidogenic cell lines examined with the highest level in JEG-3 cells; however, transcript expression was not detected in HepG2 and HK293 cells (two non-steroidogenic cell lines).

Fig. 4. The nucleotide sequence of the TReP-132 clone is shown on the top line with the first nucleotide of the most 5' cDNA denoted as 1 (SEQ ID No 1). The ATG triplets in the 5'UTR are circled. The primary structure of TReP-132 is shown on the bottom line (SEQ ID NO:2) and is represented by the single letter code. The initiator methionine numbered as 1 is in bold and the codon is denoted by an asterisk. The 3 C_2H_2 zinc finger motifs are in black boxes; the glutamine rich region, the proline rich regions and the acidic region are indicated by the open boxes. The putative polyadenylation signal is underlined.

Fig. 5. (A) Western blot analysis of both *in vitro* and *in vivo*-expressed TReP-132 protein tagged with the HA epitope at the carboxyl-end. Total protein was resolved by 8% SDS-PAGE and bands were revealed with an anti-HA polyclonal antibody. The first lane (left to right) contains HeLa cell lysate obtained from cells transfected with the TReP-132-HA cDNA in the pcDNA3 vector; the second lane is the negative control of HeLa cell lysate obtained from cells transfected with the empty pcDNA3 vector; the third lane is the negative control of *in vitro* transcribed/translated protein in the presence of the pcDNA3 vector only; and the fourth lane contains *in vitro* transcribed/translated protein produced by rabbit reticulocyte lysate from the TReP-132-HA cDNA subcloned in the pcDNA3 vector

(B) Translation of truncated TReP-132 protein from templates containing a wildtype or mutated initiator AUG codon. The TReP-132 cDNA containing the wildtype or mutated initiator codon was truncated by restriction with either Sma I or Pst I as indicated, and proteins were produced by *in vitro* transcription/translation. Mutation of the AUG codon to AUC yielded shorter protein, which is indicative of the mutated AUG codon being the initiator methionine. There are 44 residues between the initiator AUG and the next inframe AUG in the coding region. The expected number of residues in the predicted proteins are as indicated in the paragraphs on the right of the bottom panel.

Fig. 6. Interaction of TReP-132 with the human P450scc gene 5'-flanking DNA. Electrophoretic mobility shift assays were performed with a DNA probe corresponding to nucleotides -155 to -131 of the P450scc gene. Ten μg of nuclear extract protein isolated from JEG-3 cells, or the negative control of HeLa cells transfected with expression vector only (HeLa-wt), or from HeLa cells expressing exogenous TReP-132 were incubated with 200 fmol of probe (900 000 cpm) in the absence of any unlabelled probe or in the presence of a 500- and 1000-fold molar excess of unlabelled -155/-131 probe as competitor. The protein-DNA complex was clearly observed in HeLa cells expressing TReP-132; however, the complex is less apparent in JEG-3 and negative control

HeLa-wt cells, which express TReP-132 endogenously. The protein-DNA complex with labelled DNA was competed by a 500- and 1000-fold excess of unlabelled probe.

Fig. 7. Subcellular localization of TReP-132. Hela cells were transiently transfected with 2 μg of the TReP-132-HA (top panels) or the pLuc-HA expression construct for 48 hours. Cells were fixed with methanol and the immobilized proteins were incubated with the anti-HA mouse antibody followed by fluorescein, FITC conjugated anti-mouse IgG. The cells transfected with TReP-132-HA contained HA-tagged TReP-132 in the nucleus (upper right panel). The control HeLa cells transfected with pLuc-HA contained exogenous luciferase protein in the cytoplasm (lower right panel).

Fig. 8. (A) Intrinsic transcriptional activity of TReP-132. A chimeric construction composed of the GAL4 DNA-binding domain fused to the amino end of TReP-132 (GAL4-TReP-132) was generated and cotransfected into HeLa cells with 1 μg of the pFR-LUC reporter plasmid containing five GAL4 elements in front of the minimal E1b promoter. Control transfections were performed with the GAL4 DNA-binding domain alone (GAL4) or the native TReP-132 expression construct. The results are expressed as relative light units of luciferase activity. Transfections were normalized to Renilla luciferase activity, expressed from a cotransfected plasmid. Values are the mean of five independent experiments ± SD.

(B) Regulation of human P450scc gene promoter activity by TReP-132. Human placenta JEG-3 (left panel) and adrenal NCI-H295 (right panel) cells were transiently cotransfected with the –1676/+49sccLuc reporter construct and increasing amounts of the construct expressing TReP-132. The results are expressed as relative light units of luciferase activity. Transfections were normalized to Renilla luciferase activity, expressed from a cotransfected plasmid. The values are the mean of three to five independent experiments each performed in triplicate ± SD.

(C) Activation of the heterologous TK32 promoter via the –155/-131 *cis*-acting element. Human placenta JEG-3 (left panel) and adrenal NCI-H295 (right panel) cells were transiently cotransfected with the 3x(-155/-131)TK32Luc reporter construct and increasing amounts of the construct expressing TReP-132. The results are expressed as fold increase of luciferase activity over the value obtained from the empty ΔTK32 vector alone. Transfections were normalized to Renilla luciferase activity, expressed from a cotransfected plasmid. The values are the mean of three to five independent experiments ± SD.

Fig. 9. Effect of p300 and E1A on TReP-132 mediated activation of transcriptional activity. Human placenta JEG-3 cells were transiently cotransfected with 1μg of the 3x(-155/-131)TK32Luc reporter construct and either TReP-132, p300, E1A, RG2 or a combination of these proteins as indicated on the bottom of the graph. The results are expressed as fold increase of luciferase activity over the value obtained from the empty ΔTK32 vector alone. Transfections were normalized to Renilla luciferase activity, expressed from a cotransfected plasmid. The values are the mean of three to five independent experiments ± SD. p300 synergizes with TReP-132 to increase promoter activity. p300-mediated transactivation was inhibited by coexpressed E1A, but not by the E1A defective mutant RG2.

Fig. 10. (A) Interaction of TReP-132 with CBP. (Upper panel) Different regions of CBP between the amino acid residues as indicated on top of the figure were expressed as GST fusion proteins, and approximately equal amounts were immmobilized on glutathione coupled sepharose prior to incubation with the TReP-132 protein labelled with [^{35}S]methionine. The region of CBP between residues 1 to 451, 1460 to 1891 and 1892 to 2441 interacted with TReP-132, whereas the other regions did not show increased interaction above the background levels seen with incubation of TReP-132 and GST alone (GST control). The lane on the left (TReP-132 input) contains one tenth the amount of TReP-132 protein used in all incubations. Proteins were separated by 10% SDS-PAGE. (Lower panel) The same gel was stained with coomassie blue to show relatively equal loading of the CBP protein fragments.

(B) Interaction between TReP-132 and p300 in cotransfected intact HeLa cells. HeLa cells were cotransfected with plasmids expressing p300, and TReP-132 tagged with the FLAG epitope at the carboxyl-end. Following 24 hours of incubation post-transfection, an anti-p300 monoclonal antibody was used to immunoprecipitate p300 from total cell lysate. The subsequent immunoprecipitate was used in a Western blot probed with an anti-FLAG antibody. Immunoprecipitation with the anti-FLAG antibody yielded the TReP-132-FLAG protein, and immunoprecipitation with nonimmune serum yield no immunoreactive protein as expected. However, incubation of the cell lysate with the anti-p300 antisera immunoprecipitated TReP-132-FLAG, which is indicative of interaction between p300 and TReP-132.

Fig. 11. SF-1 and TReP-132 synergy is dependent on the first LXXLL motif (NR box 1) in TReP-132

A) Schematic diagram depicting deletion fragments of the carboxy-terminal domain (GAL4TRePdel1 to del4) and of the amino-terminal domain (GAL4TRePdel5 to del7) of TReP-132 fused to the DNA-binding domain of GAL4 (residues 1-147). (Top). The functional domains of TReP-132 are shown. Mutated GAL4TReP constructs and GAL4TRePdel4 in the first putative NR box LXXLL are labeled GAL4TRePm and GAL4TRePdel4m, respectively.

Additional variants: TReP-132m1 the NR box 1: SAVMDGAPDSALRQLLSQKPM is mutated to SAVMDGAPDSAARQAASQKPM; TReP-132m2 the NR box 2 LFEAKGDVMVALEMLLLRKPV is mutated to LFEAKGDVMVAAEMAALRKPV; TReP-132m1-2 both NR boxes are mutated.

B) Schematic representation of the structures of VP16SF-1 proteins used for two-hybrid assays. (Top) The functional domains of SF-1 linked to VP-16 at the amino-terminal of the protein are shown. The structure of various truncated forms of SF-1 linked to the VP16 transactivation domains are shown below. The SF-1 construct with the AF-2 hexamer deleted is labelled VP16SF-1ΔAF-2.

MATERIALS AND METHODS

1. Isolation of TReP-132 cDNA. A human placenta cDNA expression library (Cat#HL3007b, Clontech; Palo Alto, CA) was screened with multimerized copies of a double stranded oligonucleotide probe corresponding to nucleotides -155 to -131 of the human P450scc gene 5'-flanking DNA. Following hybridization of equal amounts of the strand and anti-strand oligonucleotides, they were multimerized with T4 DNA ligase and end-labeled with [α^{32}P] dATP and [α^{32}P] dGTP (NEN, Boston, MA) using Klenow fragment (New England Biolabs, Beverly, MA). The λgt11 library was screened as described (31) using a method for Southwestern blot analysis (28). After screening approximately 2 x 10^6 recombinants, three positive clones were isolated, including the initial partial TReP-132 clone (P1). A ZAP Express cDNA library was made using JEG-3 cell mRNA according to manufacturer's protocol (Stratagene, La Jolla, CA), and an additional 4 x 10^6 recombinants were screened with the P1 cDNA but yielded only 10 partial clones which did not contain the initiating codon. An additional 2 x10^6 recombinants from another human placenta λgt11 cDNA library (kindly provided by Dr. Morag Park, McGill University) were screened to isolate 6 clones including P1-CL6 which contains the putative initiator codon. To subclone the full length TReP-132 cDNA for subsequent studies, an Kpn I/Eco RI fragment from the P1-CL6 cDNA in the Bluescript KS+ vector (Stratagene, La Jolla, CA) was ligated to the Eco RI-Xba I fragment of P1-CL4 in the pcDNA3 vector (Invitrogen, Carlsbad, CA) (see Fig. 2A). Both strands of the TReP-132 cDNA were each sequenced at least twice, by dideoxy nucleotide sequencing (Amersham Pharmacia Biotech, Baie d'Urfé, Québec, Canada). The sequence has been deposited to the GenBank database and has been assigned accession number AF297872.

2. Northern Blot Analysis. JEG-3 cell total RNA was isolated by the Tri Reagent acid phenol method (Molecular Research Center Inc., Cincinnati, Ohio) and poly(A) RNA was subsequently obtained by chromatography on oligo(dT). 2 μg of mRNA was separated on a 1.1% agarose formaldehyde gel and transferred to nylon membrane (Amersham Pharmacia Biotech, Baie d'Urfé, Québec, Canada). Membrane immobilized mRNA was hybridized with radiolabeled cDNA probes (depicted in Fig. 2A) corresponding to the 5'-end of P1-CL6 and the 3'-end of P1-CL13 as previously described (29).

3. RNase protection. A radiolabeled riboprobe was generated using [α-^{32}P]UTP and the MAXIscript *In Vitro Transcription Kit* from Ambion (Austin, Texas) according to the manufacturer's protocol. The probe was generated following linearization of the TReP-132-HA cDNA in the pcDNA3 vector by digestion with Nar I, and transcription from the Sp6 promoter. The transcribed probe is a 226 bp cRNA consisting of 174 bases from the 3'-end of the TReP-132 cDNA and 52 bases from the polylinker including the HA sequences. The 18S riboprobe used for normalization was generated from the pTRI–18S-Human vector from Ambion (Austin, Texas), and produces a protected fragment of 80 bp. RNase protection assays were performed using the Ribonuclease Protection Assay II kit from Ambion (Austin, Texas), according to the manufacturer's protocol. RNA from different cells lines (20 µg) or yeast tRNA (20 µg) was hybridized to the probes at 45°C, and then digested for 1h at 37°C at a dilution of 1:75 [RNaseA/RNaseT1 Mix] in the RNase digestion buffer. The samples were resolved on a urea 6% polyacrylamide gel.

***4. In vitro* transcription/translation, coimmunoprecipitation and Western blot analysis.** Proteins were synthesized by *in vitro* transcription/translation using rabbit reticulocyte lysate (TNT coupled *in vitro* system, Promega, Maddison, WI) from plasmids containing a T7 promoter, according to the manufacturer's recommendations with [^{35}S]methionine. The radiolabeled proteins were separated by 8% SDS-polyacrylamide gel electrophoresis, and the gel was then dried and autoradiography.

To assess the apparent molecular mass of both *in vitro* and *in vivo*-expressed TReP-132 protein, whole cell lysate of HeLa cells transfected with TReP-132 tagged HA (TReP-132-HA) or with pcDNA3 alone, and the product of *in vitro* transcription/translation performed with the TReP-132-HA cDNA as template (or no template for control) were resuspended in 2X electrophoresis sample buffer. Total protein was resolved by 8% SDS-polyacrylamide gel and the gel was then tranfered to nitrocellulose membrane (Bio-Rad, Hercules, CA). Membranes were preincubated 1 hour in blocking solution containing 5% powdered milk in Tris-buffered saline (TBS), then incubated overnight at 4°C with a rabbit polyclonal anti-HA antibody (1/15000, Covance, Richmond, CA). After three washes with 0.2% Tween20 in PBS, membranes were incubated with an HRP-coupled anti-rabbit antibody (1/10000, Amersham Life Science,

Buckinghamshire, UK) for 45 min. After three washes with 0.2% Tween20 in PBS and one wash with 2% Tween20 in 10X TBS, development was performed with the Renaissance ECL Plus kit (NEN, Boston, MA) according to manufacturer's procedures.

To assess if p300 coimmunoprecipitates with p300, $1x10^7$ HeLa cells were plated on a 100 mm dish and incubated for 24 hours. Cells were transfected using 40 µl ExGen 500 per 5 µg of p300 and 5 µg of TReP-132 tagged with a Flag epitope (TReP-132-Flag). Immunoprecipitation was performed according to the procedure described by Santa Cruz Biotechnology Inc. Cells were collected in RIPA buffer (1X PBS, 0.1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitors). Cells debris were removed by centrifugation, then preincubated 30 min with normal rabbit IgG antibody (sc-2027, Santa Cruz Biotechnology, Santa Cruz, CA). The quantity of proteins in the supernatant was assessed by Bradford assay as instructed by the manufacturer (Bio-Rad, Hercules, CA). 1 mg of protein was incubated overnight at 4°C with the following antibodies: 0.1 µg monoclonal anti-Flag M2 (F3165, Sigma, St Louis, MI), 1 µg rabbit polyclonal anti-p300 (sc-584, Sigma), and 1 µg rabbit non-immune IgG for anti-p300 control. Immunoprecipitates were subjected to 8% SDS-PAGE and analyzed by Western blotting using an anti-Flag antisera (1/10 000) as the first antibody, followed by incubation with an HRP-coupled anti-mouse antisera (1/10 000, Jackson Immunoresearch Laboratories, West Grove, PA). Washes and visualisation of proteins were performed as described above.

5. Electrophoretic Mobility Shift Assays (EMSA). EMSAs were performed using double-stranded oligonucleotides containing the human P450scc gene sequence from nucleotides -155 to -131 (same as probe used for library screening). The probe was end-labeled with the Klenow fragment (New England Biolabs, Beverly, MA) in the presence of [α^{32}P] dCTP (NEN, Boston, MA). Experiments were performed using 10 µg of nuclear extract protein isolated from HeLa cells expressing exogenous TReP-132 or the negative control (vector only) or from JEG-3 cells incubated with 200 fmol (900 000 cpm) of DNA probe in the presence of 2 µg poly(dI-dC) in 20 mM Hepes pH 7.9, 60 mM KCl, 1 mM EDTA, 1 mM DTT and 5% glycerol in a final reaction volume of 20 µl for 20 min at room temperature. DNA-protein complexes were resolved by native 4% PAGE in

0.5xTris borate-EDTA for 2h at 150V. Gels were dried and exposed to film for 6 hours at -80°C.

6. Immunofluorescence and subcellular localization of TReP-132. HeLa cells ($2x10^4$) were plated on culture slides (Biocoat Culture Slides, Becton Dickinson, Flanklin Lakes, NJ, USA) and incubated for 16 hours. Transfection was then performed with Exgen 500 (MBI Fermentas, Flamborough, On, Canada) according to manufacturer's instructions. Twenty hours after transfection, cells were washed three times with cold PBS, then fixed for 15 min with Formalin (Sigma Chemical Co., St-Louis, Ma, USA). Cells were washed five times with PBS before permeabilisation with PBS containing 0.5% Triton X100 (Sigma Chemical Co.) for 15 minutes at room temperature. After three washes with PBS, cells were incubated for 1 hour in blocking buffer (6% powder milk, 3% bovine serum albumin in Tris Buffered Saline; TBS) to minimize unspecific binding of antibodies. Primary rabbit anti-HA antibodies (Covance, Richmond, CA) were added in binding buffer (blocking buffer diluted 1:10 with TBS) at 1:100 dilution. Slides were incubated overnight at 4°C and then washed twice with PBS containing 0.2% Tween 20 and twice with PBS. Secondary Alexa Fluor 594 goat anti-rabbit antibody (Molecular Probes Inc., Eugene, Or, USA) was added at 1:1000 dilution in binding buffer, and slides were incubated for 1 hour at room temperature in the dark. Washes were done as described for primary antibodies. Diamidino-2-phenylindole (DAPI) counterstain was done by incubating slides for 10 minutes in the dark at room temperature with 0.1μM dilactate DAPI (Molecular Probes Inc.). Cells were intensely washed with water, allowed to dry and were mounted as described in the Prolong Anti-Fade kit (Molecular Probes Inc.). For visualisation, a Leica DMR-B epifluorescence microscope (Leica Inc. Deerfield, Il, USA) was used in combination with standard photography. Typically, exposure times varied between 0.5-15s with a Kodak Gold 100 ASA film.

7. Tissue culture. Human NCI-H295 adrenal tumor cells, choriocarcinoma JEG-3 cells, cervical HeLa cells, and transformed primary embryonal kidney HK-293 cells were obtained from American Type Culture Collection (Rockville, MD). NCI-H295 cells were cultured in monolayer as previously described (79) in equal amounts of DMEM and Ham's F12 media (Life technologies, Gaithersburg, MD) supplemented with 1% ITS

(Roche), 1% red phenol, 10^{-8}M E2 and 10^{-8}M HOCortisone. JEG-3 and HeLa cells were cultured in MEM-red phenol medium containing 10% and 5% SVF (Hyclone, Logan, UT), respectively. HK-293 cells were cultured in DMEM containing 10% SVF. All medium were supplemented with glutamine (2mM), penicillin (55 U/ml) and streptomycin (55 μg/ml).

8. Plasmids. The reporter plasmid 3x(-155/-131)TK32Luc is comprised of three copies of the –155/-131 element generated by annealing and multimerizing the oligonucleotides: 5'GATCTCGCTGCAGAAATTCCAGACTGAACCG3' (SEQ ID NO: 3) and 3'AGCGACGTCTTTAAGGTCTGACTTGGCCTAG5' (SEQ ID NO: 4). The resulting trimerized oligo was then subcloned upstream the minimal HSV thymidine kinase promoter (TK32) into the BamHI site of the pLUC plasmid.

The P450scc luciferase reporter construct –1676/+49sccLuc contains a fragment of the human P450scc gene which span from nucleotide -1676 at the 5'-end to nucleotide +49 at the 3'-end. The DNA fragment was amplified by PCR from a P450scc genomic clone kindly provided by Dr. Bon-chu Chung (Academia Sinica, Nanking, Taipei) using oligonucleotides which introduced a Kpn I site and Bgl II site at the 5'- and 3'-end, respectively. Following amplification, the PCR product was digested with Kpn I and Bgl II and then subcloned into the pGL3 reporter plasmid (Promega; Madison, WI). Reporter constructs comprising fragments spanning –155 or –110 at the 5'-end were also prepared.

The TReP-132-HA and the pLUC-HA vectors, containing the entire cDNA of TReP-132 or luciferase tagged with the hemagglutinin (HA) epitope, were created by amplifying by PCR the TReP-132 or luciferase cDNA with oligonucleotides which introduced a Kpn I site at the 5'- and a HA- and a XbaI site and 3'-end. The PCR product were digested with Kpn I and Xba I and subcloned into the pcDNA3 expression vector (Invitrogen, Carlsbad, CA). The pcDNA3-GAL4 vector was generated by subcloning a Hind III/Xba I insert containing the GAL4 DNA binding domain (amino acids 1-147) from the pSG424 plasmid (a gift from Dr Michael R. Green, Howard Hughes Medical Institute Research Laboratories, Univ. of Mass. Medical Center, Worcester, Mass.) into the corresponding sites of the pcDNA3 expression vector. The GAL4-TReP-132 plasmid was created by subcloning a PCR product corresponding to the entire coding region of TReP-132 into the pcDNA3-GAL4 vector.

The P1 clone 2 plasmid used in GST pull-down analysis was created by inserting a PCR fragment corresponding to amino acids 751 to 1200 of TReP-132, fused in frame downstream of GST sequences in the pGEX-2TK vector (Amersham Pharmacia Biotech, Baie d'Urfé, Québec, Canada). All the constructs made were verified by dideoxy nucleotide sequencing using Sequenase 2.0 (Amersham Pharmacia Biotech, Baie d'Urfé, Québec, Canada).

The pFR-LUC plasmid, which is comprised of five GAL4 elements upstream of a minimal E1b TATA box followed by the luciferase reporter gene, was purchased from Stratagene (La Jolla, CA). Plasmids encoding p300 were kindly provided by Dr. Richard H. Goodman (Oregon Health Sciences University) and plasmids encoding E1A and the RG2 mutant as described by Wang *et al.* (96) were kindly provided by Dr. Claude Labrie (CHUL Research Center). Plasmid encoding GST fusion protein with CBP fragments were provided by Dr. Ralf Janknecht (The Salk Institute for Biological Studies) (36).

9. Transfections and Luciferase assay. HeLa and HK-293 cells were cultured in 12 well plates at an initial density of $1x10^5$ cells per well; and JEG-3 and NCI-H295 cells were plated at a density of $3x10^5$ cells per well. Following 24 hours of incubation the medium was changed and transient transfections were carried out for 12h. HeLa, HK-293 and JEG-3 cells were transfected with ExGen 500 (MBI Fermentas, Flamborough, Ontario, Canada) at a ratio of 8 µl ExGen 500 per 1.5 µg of DNA. NCI-H295 cells were transfected with Effectene Transfection Reagent (Qiagen, Mississauga, Ontario) at a ratio of 1 µg of DNA to 25 µl of Effectene. Following transfection for 12 hours the cells were incubated in fresh medium for 12 to 36 hours.

For luciferase assays, cells were lysed by the addition of 250 µl of lysis buffer (0.8% Triton-X100, 25 mM glycylglycine pH7.8, 15 mM MgSO4, 4mM EGTA) and incubated for 15 minutes. Fifteen µl of the cell lysate was assayed using the Luciferase Assay System from Promega Corp. (Madison, WI) in a Berthold LUMAT LB9501 luminometer. All experiments were normalized by cotransfecting 0.1 µg of a β-galactosidase expression plasmid driven by the Rous sarcoma virus promoter. β-Galactosidase assays were performed using 10 µl of lysate in the Galacto–Light Plus assay system (Tropix, Bedford, MA).

For the transfection of each cell type, the efficiency of transfection was verified by a GFP expression plasmid, and the efficiencies obtained were 60% or better.

10. In Vitro **Protein Binding Assay.** To ascertain the interaction between CBP/p300 or SF-1 and TReP-132 *in vitro*, fragments of CBP or SF-1 polypeptides from different regions of the proteins were expressed as GST fusion proteins and were immmobilized on glutathione coupled sepharose as described by Frangioni and Nell (17) prior to incubation with radiolabelled TReP-132 protein. [^{35}S] labelled TReP-132 protein was produced from the cDNA (as described above) using rabbit reticulocyte lysate and T7 RNA polymerase (Promega). GST-CBP and GST-SF1 fusion proteins were produced from cDNA constructs in *E. coli* BL21 following induction with 0.1 mM isopropythiogalactopyranoside. Equal quantities of GST-CBP fusion proteins were incubated with glutathione coupled sepharose for 30 min at 4°C in binding buffer (100mM NaCl, 1mM EDTA pH 8, 20mM Tris pH 8.0, 0.5% NP-40). The beads were then washed 3 times with PBS and once with binding buffer, and were then incubated with radiolabeled TReP-132 for 2 hours at 4°C in the same buffer. After incubation, the beads were washed five times with washing buffer (20 mM Tris-Cl pH 8, 500 mM NaCl, 5 mM EDTA pH 8.0 and 0.1% Triton X-100). Bound proteins were released from the sepharose by boiling in SDS sample buffer and were analyzed by SDS-PAGE (48). The gels were stained with coomassie blue to ascertain that equal amounts of GST proteins were loaded, after which the gels were incubated for 30 min with Amplify (Amersham; Oakville, Ont), dried and exposed to film.

RESULTS

11. Isolation of TReP-132 cDNA clone. The promoter region of the human P450scc gene contains a sequence between nucleotides -155 and -131 (-155/-131) that was previously demonstrated to confer increased transcriptional activity when fused to a heterologous HSV thymidine kinase minimal promoter (TK32). Three copies of this element conferred significant activation of TK32 promoter activity of 500- and 80-fold when the reporter construct was transiently transfected in human placental JEG-3 (28) or human adrenal NCI-H295 cells, respectively (Fig. 1). However, promoter activation was

significantly less in non-steroidogenic cervical HeLa cells and was not observed in kidney HK293 cells. In a previous study, this element was also demonstrated to interact specifically with at least two proteins in JEG-3 cell nuclear extract as determined by electrophoretic mobility shift assay (EMSA) (28, 66). To isolate cDNA clones which encode these putative transcription factors, a human placenta cDNA expression library was screened with multimerized copies of the -155/-131 DNA fragment, and a positive clone (P1) of 1.6 kb was isolated (Fig. 2A). The P1 cDNA has a coding sequence in-frame with the β-galactosidase fusion protein in the λgt11 cloning vector. The open reading frame in P1 encodes 451 residues with a primary structure that contains an acidic region, a region rich in prolines and two zinc-finger motifs, which suggest the isolation of a DNA binding transcription factor. Expression of the cDNA by coupled *in vitro* transcription/translation in rabbit reticulocyte lysate produced a protein of approximately 52 kDa, in agreement with the predicted coding sequence (data not shown).

Northern blot analysis on JEG-3 cell mRNA probed with the 1.6 kb P1 cDNA, revealed two hybridizing transcripts of approximately 4.4 and 7.5 kb (data not shown). To isolate the full-length cDNA, the initial library and a subsequent human placenta cDNA library (kindly provided by Dr. Morag Park, McGill University) was screened using the 1.6 kb P1 cDNA as probe to obtain a clone which contains the entire coding region. From analysis of the 22 clones isolated (of which only a subset is depicted in figure 2A), it is apparent that two classes of clones were isolated. Based on the sizes of the clones and the overlap of sequences, it can be deduced that some clones contain a 3'-untranslated region, which is approximately 2.5 kb longer. To determine if different lengths of the 3'-untranslated region can explain the two bands seen in Northern blots, a probe from the 5' end of clone P1-Cl4 hybridized to the same sized transcripts of 4.4 and 7.5 kb as seen with the P1 cDNA, whereas a probe from the 3' end of clone P1-CL13 hybridized only to the transcript of 7.5 kb (Fig. 2B). As well, PCR amplification of the 3' end of clones P1-CL13 and P1-CLF using a 5' primer immediately upstream of the poly (A) tail in P1-Cl4 and a 3' primer immediately upstream of the poly (A) tail of P1-CL13 yielded a product of 2 kb (data not shown), thus indicating that this region accounts at least in part for the larger transcript observed in Northern blots. In support of the coexistence of these two transcripts which may arise from differential polyadenylation sites, a consensus adenylation signal AATAAA is found 20 and 21 nucleotides upstream of the poly (A) tail

in clone P1-CL4 and P1-CL13 respectively. In addition, all the cDNA clones which were isolated with the P1 probe have an open reading frame which terminates at the same stop codon regardless of the length of the 3'-untranslated region.

12. Tissue distribution of TReP-132 transcripts. The TReP-132 cDNA clones were isolated from human placenta libraries; however, cytochrome P450scc is expressed in additional steroidogenic tissues. To determine the distribution of TReP-132 transcript expression in different human tissues, Northern blot analysis was performed using a 504 bp cDNA probe from nucleotides 532 to 1035 (Fig. 3A). Both the 4.4 and 7.5 kb transcripts were detected in most of the tissues examined except for the pancreas and small intestine, which exhibited very low levels. However, it is clear that the thymus, testis and adrenal cortex express the highest levels of both transcripts. To ascertain the expression of TReP-132 transcript in established cell lines, RNase protection assays were performed with a probe of 226 bp derived from the 3'-end of the cDNA clone. As found in the tissues, varying levels of transcript are expressed in the cell lines examined. However, expression is most prominent in the steroidogenic JEG-3 and MCF-7 cells, and no expression was detected in the non-steroidogenic HepG2 and HK293 cells (Fig. 3B).

13. Primary structure of TReP-132. The open reading frame of TReP-132 is comprised of 3600 nucleotides, which encode a polypeptide of 1200 residues with a predicted molecular mass of 132 kDa. Analysis of the primary structure of TReP-132, which was predicted from nucleotide sequence, revealed several motifs which suggest the function of this protein as a DNA binding transcription factor (Fig. 4, SEQ ID NOs: 1 and 2). The region between residues 252 to 343 contains several glutamine rich sequences, the regions between residues 553 to 570 and 984 to 1009 contain many prolines, and the region between amino acids 956 to 982 contains 17 glutamate residues, all of which have been found in other proteins to be involved in transcriptional activation. TReP-132 contains three C_2H_2-type zinc finger motifs, which in other proteins have been shown to be involved in protein interaction and DNA binding. Two sequences LRQLL and LEMLL are also found, which match the canonical LXXLL sequence that has been implicated in interaction with nuclear receptors. Based on the primary structure of TReP-132 it would

be reasonable to predict that the protein interacts with other factors to regulate gene transcription.

When expressed *in vitro* using rabbit reticulocyte lysates, the P1-CL4 cDNA encodes a protein with an apparent molecular mass of 132 kDa, which is in agreement with the predicted size (data not shown). Expression of the full length TReP-132 cDNA, which contains 563 bp of 5'-untranslated sequence from P1-CL6 in addition to all of the sequence in P1-CL4, yielded a protein of identical size suggesting the usage of a common initiator AUG codon found in both P1-CL4 and full length TReP-132 (Fig 5A). The first ATG in P1-CL4 is in-frame with the putative coding sequence, thus suggesting that it is the initiating methionine of the protein. In TReP-132 there are 10 other ATG codons upstream of the putative first codon; however, any translation initiating from them would lead to short open reading frames. Considering the unusually long 5'-untranslated region of this clone, the issue of identifying the initiator codon was further confirmed by mutation of the predicted initiator AUG to AUC in TReP-132. Prior to expression, the mutated cDNA was digested at the Sma I or Pst I site in the coding region to yield a shorter protein to facilitate detection of changes in protein size by SDS-PAGE. Subsequent expression of the mutated and truncated TReP-132 cDNA yielded a shorter protein, which is indicative of the initiator codon being mutated, and translation initiating at a downstream internal ATG (Fig 5B). Transient expression in HeLa cells of TReP-132 tagged with the HA-epitope produced a 132 kDa protein that comigrates in SDS-PAGE with the protein produced *in vitro*; thus there is no apparent indication of a gross post-translational modification leading to altered mobility when the protein is expressed *in vivo* (Fig. 5A).

Since the TReP-132 clone was isolated by screening a cDNA expression library with the –155/-131 sequence, it would be expected for this protein expressed in HeLa cells to bind the DNA element. In EMSAs, nuclear extract from HeLa cells expressing TReP-132 produced a significant protein-DNA complex. Complex formation with the radiolabeled probe was competed by incubation with an excess of unlabelled probe; and the specific complex was significantly less apparent when using nuclear extract from HeLa or JEG-3 cells transfected with the empty expression vector only (Fig. 6). This is consistent with endogenous TReP-132 in HeLa and JEG-3 cells forming a limited amount of complex with the DNA probe, and the overexpressed exogenous TReP-132 protein in

HeLa cell nuclear extract forming more of the protein/DNA complex. In agreement with this, RNase protection analysis demonstrate the expression of endogenous TReP-132 in HeLa and JEG-3 cells.

14. Subcellular localization of TReP-132 protein. Considering that TReP-132 is a putative DNA-binding protein, fluorescent immunocytochemistry was performed to establish its subcellular localization. A construct that expresses a chimeric TReP-132 protein with an HA-tag at its carboxyl terminus was transiently transfected into HeLa cells, and subcellular localization of the protein was detected by an anti-HA antibody. In all the transiently transfected cells, the tagged protein was clearly localized in the nucleus (Fig. 7). HeLa cells which were stably transfected, also contained the chimeric TReP-132 protein in the nucleus (data not shown).

15. Effect of TReP-132 on human P450scc gene promoter activity. To determine if TReP-132 has an intrinsic activity to regulate transcription, a chimeric protein was generated by fusion of the Gal4 DNA-binding domain (DBD) to the amino-terminus of full length TReP-132. Transient expression of this protein was able to significantly increase promoter activity of a reporter plasmid controlled by five Gal4-binding sites upstream of the E1b minimal promoter. This activation was not observed with expression of the Gal4 DBD or TReP-132 proteins alone, thus demonstrating the ability of the DNA interacting Gal4-TReP-132 fusion protein to specifically activate transcription (Fig. 8A).

To determine if TReP-132 can affect promoter activity of the P450scc gene, the cDNA subcloned in pcDNA3 was cotransfected transiently with a luciferase reporter construct (–1676/+49sccLuc) in human placental JEG-3 and adrenal NCI-H295 cells. The 5'-flanking region of the human P450scc gene from nucleotides -1676 to +49 conferred basal promoter activity in both cell types; and coexpression of the 132 kDa protein had a dose dependent stimulatory effect, which activated transcription in JEG-3 and NCI-H295 cells (Fig. 8B). To determine if TReP-132 can activate promoter activity via the –155/-131 element, it was shown that cotransfection of the cDNA increased expression of the 3x(-155/-131)TK32Luc reporter plasmid in a dose dependent manner 25- and 3.5-fold in JEG-3 and NCI-H295 cells, respectively (Fig. 8C).

16. Interaction of TReP-132 with CBP/p300. Several well-characterized transcription factors have been shown to interact with the coregulators CBP/p300 to regulate gene expression. Thus, to ascertain if TReP-132 can functionally interact with the coregulators, it was coexpressed with p300 in JEG-3 cells. Expression of TReP-132 or p300 separately, increases expression of 3x(-155/-131)TK32Luc. However, the coexpression of both proteins interact synergistically to further promote expression via the –155/-131 element (Fig. 9). Similar results were also obtained in NCI-H295 cells (data not shown). The specificity of p300 induced activation was demonstrated by co-expression of E1A with TReP-132, which inhibited the effect of p300. As well, the co-expression of R2G (E1A mutated at residue 2, which abolishes interaction with p300) with TReP-132 did not effect the activating function of p300.

To determine if TReP-132 physically interacts with CBP/p300, GST fusion proteins, which contain different regions of CBP, were bound to glutathione sepharose and incubated with ^{35}S labeled protein produced from the TreP-132 cDNA. The protein encoded by TreP-132, which contains the residues from 1 to 1200 of TReP-132 interacts with the region from amino acids 1 to 451 and 1460 to 1891 and 1892 to 2441 of CBP, but no interaction was observed with the regions between residues 451-721, 721-1100 and 1099-1460 (Fig. 10A).

To assess if TReP-132 interacts with p300 in intact cells, expression plasmids encoding p300, and TReP-132 tagged with the FLAG epitope were cotransfected into HeLa cells. Immunoprecipitation of p300 from total cell extract using an anti-p300 antisera followed by Western blot analysis using an anti-FLAG antibody revealed the interaction between these two proteins *in vivo* (Fig. 10B).

17. TReP-132 and SF-1 synergies for activation of the P450scc promoter

To determine the ability of TReP-132 to mediate SF-1 regulation of the human P450scc gene, luciferase reporter constructs –1676Luc, -155Luc and -110Luc were cotransfected with SF-1 and TReP-132. Transcription were enhanced by about 2 fold above luciferase activity conferred by SF-1 and by 2 to 4 fold above luciferase levels conferred by TReP-132 alone. Mutation of the SF-1 binding site at nucleotides –38 to –46 in the three P450scc promoter constructs abolished synergy of SF-1 and TReP-132.

Thus TReP-132 modulates SF-1 activation at the -46-SF-1 binding site.

18. TReP-132 and SF-1 interact directly

In vitro interaction: To assess whether TReP-132 and SF-1 interact directly, we used the GST pull-down assay. An immobilized gluthatione-S-transferase (GST)-SF-1 fusion protein specifically interacts in vitro with 35S-labeled TReP-132 whereas GST alone did not, indicating that the GST-SF-1 interaction is not mediated by GST alone. Reciprocal results were obtained when TReP-132 was linked to GST and was shown to interact specifically with labelled SF-1.

In vivo interaction: To investigate if TReP-132 can interact with SF-1 directly in the cell, immunoprecipitation assays were performed. TReP-132 tagged with a Flag epitope (TReP-132-Flag) and SF-1 were cotransfected in HeLa cells. Potential complexes were immunoprecipitated using a polyclonal anti-SF-1 antibody and subjected to western blot analysis using a monoclonal anti-Flag antibody. The results show that TReP-132-Flag was detected by the anti-Flag antibody in the immunocomplex precipitated with the anti-SF-1 antibody.

To confirm by another method that SF-1 can interact with TReP-132 in vivo, two-hybrid assays were carried out in HeLa cells. TReP-132 fused to the GAL4 DNA-binding domain (TReP-GAL4) and SF-1 fused to the transactivation domain of VP16 (VP16-SF-1) were cotransfected with the PFRLuc reporter plasmid containing 5 response elements to GAL4. The coexpression of both proteins yielded significantly higher activity than cotransfection of SF-1-VP16 with GAL4 or VP-16 with TReP-132-GAL4.

19. Interaction of TReP-132 with SF-1 involves the NR box LRQLL of TReP-132

In order to investigate which domains of TReP-132 interacts with SF-1, we employed in addition to the GAL4TReP construct which contains the full length coding region of TReP-132, seven GAL4 fusion proteins encoding various fragments of TReP-132 deleted at the carboxy- or amino-region of the protein. The GAL4TRePdel4 construct that was mutated in the first LRQLL motif (leucine were changed in alanine) of the protein was also constructed (Figure 11A). These constructs and pcDNA3GAL4 were cotransfected with VP16 or VP16-SF-1 into HeLa cells along with pFRLuc. Coexpression of both GAL4TReP and VP16SF-1 generates increased luciferase activity above activity obtained

by VP16-SF-1 cotransfected with GAL4, or by GAL4TReP cotransfected with VP16. Cotransfection of the truncated/mutated GAL4TReP fusion proteins with VP16 didn't change luciferase activity compared to the expression of the proteins with pcDNA3. Three truncated fusion proteins, GAL4TRePdel2 to del4, which contain deletions at the carboxy-terminal activated transcription of the pFRLuc reporter plasmid. Co-expression of VP16-SF-1 with these GAL4 fusion proteins stimulated luciferase activity, higher than those observed with GAL4TReP del2 to del4 alone for each of these constructs. Surprisingly, deletions of the two zinc fingers in the carboxy-terminal region of the protein led to a decrease of the luciferase activity, when plasmid was cotransfected with VP16 or with VP16-SF-1. These results suggest that the carboxy-terminal domain and notably the zinc fingers, glutamine and proline rich regions of TReP-132 are regulator elements of transcriptional activation and can influence SF-1 interaction.

The fusion proteins deleted in the amino-terminal domain of TReP-132 were unable to activate transcription (GAL4TReP del 5 to del 7). Mutation of the LRQLL motif in the full length GAL4TReP construct and in the truncated GAL4TRePdel4 construct abolished totally the transcriptional activity of both proteins. These double-hybrid assays indicate that in the context of the full length TReP-132 protein, the LRQLL motif is necessary for the TReP-132 basal activation and for interaction with SF-1, and that the other motifs, which include the two carboxy-terminal zinc fingers, the proline, the glutamine and glutamic acid rich regions, have regulatory roles.

To test whether the LRQLL motif is also the motif involved in the interaction with SF-1 for P450scc gene activation in NCI-H295 cells, leucines of the motif were mutated to alanines in the TReP-132 construct cloned in pcDNA3 (the mutated construct was denoted TReP-132m1). When TReP-132m1 was transfected in NCI-H295 cells, the basal activation conferred on the –110 construct was decreased, and synergy with SF-1 was abolished. When TReP-132 was mutated in the LEMLL motif, which is the second NR box of TReP-132 (TReP-132m2), basal transcriptional activity and synergy with SF-1 was conserved, although they were reduced (figure 11B). Mutation of both NR boxes (TReP-132m1-2) conferred a lost of luciferase activity, as with TReP-132m1, confirming that the LRQLL motif is involved in interaction with SF-1.

20. TReP-132 interacts with SF-1 and potentiates its activity through AF-2-AH and an additional domain, which is not the PAD involved in SRC-1 interaction

Nuclear hormones receptors such as the thyroid hormone receptor (TR), estrogen receptor (ER), and retinoic acid receptor (RAR) are known to contain a transactivation domain within the carboxy-terminal region of the receptors. A conserved motif of six amino acids (called AF-2 hexamer), previously identified as a conserved activation domain in several steroid receptors is found at the carboxy-terminal of SF-1. This motif is composed of glutamate, preceded by a variable amino acid, flanked by two pairs of hydrophobic amino acids. Furthermore, a proximal activation domain (PAD1) located between the nucleotides 187 to 245, is involved in interaction with SRC-1. To test whether the AF-2, PAD or others domains of SF-1 are required for interaction with TReP-132, a mammalian two-hybrid system was performed in HeLa cells, using a series of deletion fragments of the carboxy terminal domain of SF-1, fused to the activation domain VP16 (called VP16-SF-1del) cotransfected with GAL4TReP or with GAL4TRePdel4 as above. Deletions of the nucleotides 245 to 462 and of the AF-2 domain in the fusion constructs (VP16-SF-1del4 and VP16-SF-1ΔAF-2, respectively), lead to the loss of functional interaction between SF-1 and TReP-132, thus TReP-132 interactions with SF-1 are mediated through the AF-2 domain. Cotransfection of the VP16-SF-1-del1 construct, which contains the nucleotides 245 to 462, reduced drastically luciferase activity, showing that the AF-2 domain of SF-1 is necessary but not sufficient to confer SF-1 activity. Conservation of the PAD1 domain (positionned between nucleotides 187 to 245) in the VP16-SF-1del fusion protein (VP16-SF-1del4) restored some luciferase activity. Total luciferase activity was obtained with coexpression of the VP16-SF-1del3 construct, which contains the carboxy terminal domain from nucleotides 118 to 462. Thus the amino-terminal region between residues 118 and 187, in addition to the AF-2 domain, is required for potentiation by TReP-132.

DISCUSSION

The regulation of P450scc gene expression is a key determinant of steroid synthesis in steroidogenic tissues, considering that the encoded cytochrome P450scc catalyzes the first step in the steroid synthesis pathway. The tissue specific expression of

P450scc transcript (11, 62) and its regulated expression in response to peptide hormones (61, 82, 98), demonstrate the importance of transcriptional regulation on P450scc levels. Previous analysis of the 5'-flanking region of the human P450scc gene isolated a *cis*-acting element between nucleotides –155 and –131, which activated transcription from the P450scc gene promoter and from the heterologous hsv thymidine kinase gene promoter, when reporter plasmids were transfected into human placental JEG-3 cells. As well, proteins from JEG-3 cell nuclear extract interacted with the –155/-131 element to form protein/DNA complexes in EMSAs (28, 66). To characterize the transcription factors, which are potentially involved in P450scc gene regulation, human placenta and JEG-3 cell cDNA expression libraries were screened in the present study to isolate clones which interact with the –155/-131 *cis*-acting element. One first clone P1 was isolated based on DNA interaction, and subsequent expression of the full length TReP-132 cDNA in HeLa cells confirmed interaction of this protein with the –155/-131 DNA fragment by EMSA.

Nucleotide sequence analysis of the TReP-132 cDNA clone revealed an open reading frame of 3600 bases preceded by a 5' untranslated region (5'UTR) of 563 nucleotides, which contains 10 ATG sequences prior to the putative initiator codon. However, the nucleotide context of the predicted initiator AUG (GCAGACAGCAUGG) is the most homologous to the consensus sequence GCCGCC(A/G)CCAUGG for initiator codons, as defined by Kozak (41, 44, 46). Eight of the 10 upstream open reading frames (uORFs) in the 5'UTR also provide a termination site before the main ORF; and the ninth uORF has a termination site TGA found 3 nucleotides after the initiator codon. Since 9 of the 10 ATGs in the 5'UTR lie in poor sequence contexts for translation initiation, it is possible that they are inefficiently recognized or ignored by scanning ribosomes (42, 45). However, it is also possible that these upstream ATGs are recognized by scanning ribosomes, and in which case can modulate initiation of the main ORF (3, 43, 47). Alternatively, as has been shown for S-adenosyltransferasemethionine decarboxylase (18, 80) and yeast GCN4, peptides from the uORF can modulate initiation of translation. To confirm that the main ORF of TReP-132 starts at the predicted initiator AUG, mutation of this codon in the context of the 5'UTR led to translation of a shortened protein.

Upon analysis of the different cDNA clones isolated during the screening process to obtain full length TReP-132, it was apparent that there exists at least two types of

TReP-132 transcripts, which differ in length at the 3'UTR. The shorter of the 3'UTRs is 217 nucleotides in length between the stop codon and the poly(A) tail, and the other is approximately 2.5 kb longer (data not shown). In both cases, a consensus nuclear polyadenylation signal is found at the appropriate position upstream of the poly (A) tail. On Northern blot analysis, a cDNA probe from the coding region hybridized to two transcripts of approximately 4.4 and 7.5 kb. However, a probe from the longer 3'UTR hybridized only to the 7.5 kb mRNA, which indicates that this region accounts for at least part of the longer transcript. The 3'UTR of several transcripts have been shown to be involved in modulating mRNA stability as well as effecting the efficiency of translation (86, 101), thus it will be interesting to decipher the role of the two 3'UTRs in the TReP-132 transcripts. Considering the existence of different 3'UTRs and the usual length of the 5'UTR, it is tempting to speculate that the two ends in the TReP-132 transcripts may interact to effect the efficiency of translation (12, 21, 77). Northern blot analysis of mRNA from different human tissues show differential levels of the two TReP-132 transcripts; however, the physiological relevance of two transcripts of different lengths encoding TReP-132 remain to be elucidated.

During the screening process to isolate the full length TReP-132 cDNA, a partial clone that was missing a region of the 5'-end was isolated, but which also had a deletion of the codons encoding residues 546 to 628. A recent examination of the GenBank database revealed the homology of TReP-132 with a sequence (accession #AJ277275) which is identical except for the extra sequences in TReP-132 encoding the residues 546 to 628. Thus, it is apparent that there are isoforms of TReP-132; however, the functional and physiological significance of these will require further studies. As well, it is tempting to speculate that the presence of the insert in TReP-132 is the result of alternative splicing of pre-mRNA.

Transient expression of exogenous TReP-132 increased expression of the –1676/+49sccLuc reporter plasmid in a dose dependent manner in JEG-3 and NCI-H295 cells, indicating the ability of this protein to activate P450scc gene transcription. Transient transfection of the TReP-132 cDNA in these cells also significantly increased, in a dose dependent manner, the expression of the 3x(-155/-131)TK32Luc reporter plasmid; thus demonstrating that the –155/-131 element confers response to TReP-132. It is interesting that the magnitude of reporter activation by exogenous TReP-132 is

consistently higher in JEG-3 than NCI-H295 cells, which correlates with the higher basal activity of the reporter plasmids in JEG-3 cells in the absence of the exogenous protein. The fusion of TReP-132 with the DNA-binding domain of the Gal4 protein was also able to activate transcription from the E1b promoter, via interaction with upstream Gal4 binding sites; thus demonstrating the intrinsic activity of TReP-132 to regulate transcription. These results along with the subcellular localization in the nucleus, indicate this novel protein as a transcription factor.

Analysis of the TReP-132 primary structure of 1200 residues revealed sequences, which further support the role of this protein as a transcription factor. The protein contains three zinc finger motifs of the C_2H_2 type, which is found in many transcription regulatory proteins (39) including Sp1 (63), WT1 (71), NGFI-A (50), MBP-1 (16), MBP-2 (94) and Krox-20 (78), and have been demonstrated to interact with DNA. The zinc finger motifs are usually found as concatemers in distinct domains in many proteins; however, TReP-132 contains a single motif in the central region of the protein and two at the carboxyl terminus. Interestingly, polypeptides with only one (75) or two zinc fingers have also been demonstrated to complex with DNA (15). In addition to DNA binding, domains containing C_2H_2 zinc fingers have been implicated in nuclear localization (56), protein-protein interaction (40, 59) and DNA bending (84). Interestingly, mutations in the C-terminal zinc fingers of the Ikaros proteins, which are also of the C_2H_2 subtype, ablate Ikaros protein interactions and decrease the ability of these proteins to bind DNA and activate transcription (88). In addition to the zinc finger domains, TReP-132 contains motifs which have been implicated in transcriptional activation in other proteins. A large region between amino acids 262 to 343 is rich in glutamine residues, which has been shown in several proteins including Sp1 (63) and Oct-1 (91) to confer activation of transcription. The region between residues 553 to 574 is proline-rich, which has been shown to form an important activation domain in CTF (60) and Oct-2 (90); and the region between 956 to 1009 contains acidic residues and is rich in prolines, where such a domain is shown to be important in p53 function (93). These characteristic regions, which form activation domains in various proteins, have been shown to mediate increased transcription by interacting with basal transcription proteins of the RNA polymerase II protein complex or with other intermediary transcription factors. The presence of these

sequences in TReP-132 suggests its potential interaction with other proteins, required to regulate gene expression.

It has recently been shown that CBP/p300 is involved in the regulation of human P450scc gene promoter activity (64), thus the present study also addressed the potential interaction between TReP-132 and CBP/p300 involved to regulate gene expression. CBP and p300 are ubiquitous transcriptional integrator proteins, which have been demonstrated to mediate the activity of many factors such as MyoD (103), AP-1 (38), SRC-1 (85, 102) p65 (19), and the nuclear receptors ER (23), RAR, RXR, and TR (4, 35). The coexpression of exogenous TReP-132 and p300 in JEG-3 cells demonstrated a synergistic activation of the 3x(-155/-131)TK32Luc reporter plasmid, which is indicative of a functional interaction between the two proteins to regulate P450scc gene expression via the –155/-131 element. Human p300 was identified initially by its ability to bind the adenoviral E1A oncoprotein (68) that can transform primary cells, block cellular differentiation and inhibit certain transcriptional enhancer elements. Binding of E1A to CBP/p300 has been shown to abolish or downregulate the stimulatory effects of c-Fos (2), cJun, JunB (49), MyoD (103) and CREB (1, 54). In this study, the specificity of activation by p300 was ascertained by cotransfection of E1A, which inhibited the effects of exogenous p300 and TReP-132 expressed individually or in combination. The positive effect of only TReP-132 alone on 3x(-155/-131)TK32Luc was also inhibited by E1A, which most likely is mediated by inhibition of endogenous p300. In agreement with previous studies, which show that mutation of E1A at position 2 can abolish interaction with p300 and alleviate its inhibitory effect, RG2 is shown to be unable to inhibit the stimulatory effect of TReP-132 and p300 on 3x(-155/-131)TK32Luc expression. The results of GST pull-down and immunoprecipitation analyses further demonstrate the interaction between TReP-132 and CBP. In pull-down experiments, the region of TReP-132 from residues 439 to 1200 (P1-CL2) interacts with the region of CBP between residues 1460 and 1891, which has also been shown to interact with numerous other transcription factors including SF-1 (20, 64).

In addition to having domains potentially implicated in DNA binding and activation of transcription, TReP-132 also has several regions rich in leucine residues including two LXXLL sequences which has been proposed to interact with nuclear receptors. It has been reported that the proteins including p/CIP, NCoA-1, NCoA-2, RIP-

140, SRC-1, CBP and RAC3, which interact with nuclear receptors, contain a consensus core LXXLL sequence motif in their nuclear receptor interaction domains (25, 57, 65, 76, 92, 100). The presence of the LXXLL sequences in TReP-132 suggests that it can also interact with nuclear receptors such as SF-1, which has been demonstrated to regulate P450scc gene expression (5, 10, 13, 37, 52, 53, 55, 64, 81). Thus, with the recent finding that SF-1 interacts with CBP/p300 (34, 64), and the present demonstration of TReP-132-CBP/p300 interaction, it is tempting to speculate on the interaction between these three factors. Considering that nuclear receptor-associated coactivators such as SRC-1 (72), TIF2 (95) and RAC3 (51) are capable of mediating the activity of several receptors, it is possible that TReP-132 can associate with different nuclear receptors to mediate gene expression. It has been postulated that the negative cross-talk observed between nuclear hormone receptors and AP-1, which all interact with CBP/p300, may be the result of competition for interaction with limiting amounts of integrator protein (38). However, recent studies have also indicated a role of CBP/p300 for mediating the positive cross-talk between nuclear hormone receptors and p45/NF-E2 (7), as well as, mediating the activity of SRC-1 to enhance steroid receptor-dependent transcription (23, 85). Similarly to SRC-1, which interacts with CBP/p300 and ER to synergistically stimulate receptor-dependent transcription, an interaction of TReP-132 with SF-1 and CBP/p300 may be a mechanism by which this complex can exert an effect on gene expression.

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CLAIMS

1. Use of a TReP-132 polypeptide or a nucleic acid molecule encoding the same, for the in vitro selection of compounds that regulate gene expression.

2. Use of a TReP-132 polypeptide or a nucleic acid molecule encoding the same, for the manufacture of a composition suitable to regulate gene expression in vivo.

3. Use of a compound that modulates expression or activity of a TReP-132 polypeptide, in the manufacture of a medicament for treating steroid hormone-related disorders or lipid disorders, particularly steroid-dependent cancers, cardiovascular diseases and obesity.

4. The use according to claim 3, wherein the compound stimulates or represses TReP-132 expression or activity.

5. The use according to claim 3, wherein the compound has a TReP-132-like activity.

6. The use according to any one of claims 3 to 5, wherein the compound is a nucleic acid, a peptide, an antibody or a synthetic molecule.

7. A method of screening, selecting or identifying compounds that regulate gene expression, the method comprising contacting in vitro a candidate compound with a TReP-132 polypeptide, and selecting the candidate compound that binds said TReP-132 polypeptide, the binding to a TReP-132 polypeptide being indicative of a gene expression regulation activity.

8. A method of screening, selecting or identifying compounds that regulate gene expression, the method comprising contacting in vitro a candidate compound with a TReP-132 receptor in the presence of a TReP-132 polypeptide, and selecting the candidate compound(s) that modulate(s) the binding of said TReP-132 polypeptide to said receptor, said modulation being indicative of a gene expression regulation activity.

9. The method of claim 8, wherein the receptor is a nuclear receptor, a co-factor, a binding partner or nucleic acid molecule to which TReP-132 binds or with which TReP-132 interacts, or a part thereof comprising the binding site.

10. The method of claim 9, wherein the receptor is a nucleic acid molecule comprising the sequence SEQ ID NO: 3 or 4 or 5 or a complementary sequence or a functional variant thereof, or all or part of a CBP/p300 or of a SF-1 polypeptide.

11. A method of screening, selecting or identifying compounds that regulate gene expression, particularly expression of a gene involved in steroidogenesis, the method comprising contacting a candidate compound with a cell comprising a reporter construct, said reporter construct comprising a reporter gene under the control of a promoter comprising SEQ ID NO: 3 or 4 or a functional variant thereof, and selecting the candidate compound that modulates expression of the reporter gene, said modulation being indicative of a gene expression regulation activity.

12. The method of claim 11, wherein the contacting is performed in the presence of a TReP-132 polypeptide, and the compounds that modulate expression of the reporter gene are selected.

13. A method of screening, selecting or identifying compounds that regulate gene expression, particularly expression of a gene involved in steroid synthesis, the method comprising contacting a candidate compound, in the presence of a TReP-132 polypeptide, with a cell comprising a reporter construct, said reporter construct comprising a reporter gene under the control of an SF-1-responsive promoter, and selecting the candidate compound(s) that modulate(s) (e.g. stimulate(s) or reduce(s)) expression of the reporter gene, said modulation being indicative of a gene expression regulation activity.

14. The method of claim 13, wherein the SF-1-responsive promoter is a promoter comprising one or several copies of SEQ ID NO: 5 (TCAAGGCCA) or a complementary sequence or a functional variant thereof.

15. The method of claim 13 or 14, wherein the cell contains or expresss a SF-1 polypeptide.

16. A recombinant nucleic acid molecule comprising a promoter and several copies of SEQ ID NO: 3 or 4 or 5 or a functional variant thereof.

17. A recombinant nucleic acid comprising all or a transcriptionally functional part of a promoter selected from viral promoters and mammalian promoters, fused to one or several copies of SEQ ID NO: 3 or 4 or 5 or a functional variant thereof.

18. A polypeptide comprising :

. the amino acid sequence of SEQ ID NO: 2, or

. amino acid sequence from residues 546 to 628 of SEQ ID NO: 2, or a fragment thereof comprising at least 4 consecutive residues thereof, or

. amino acid sequence from residues 1 to 161 of SEQ ID NO: 2, or a fragment thereof comprising at least 4 consecutive residues thereof, or

. a 200 amino acid or less fragment of a TReP-132 polypeptide comprising a LRQLL region or a LEMLL region or a Zinc Finger motif, or

. a TReP-132 polypeptide lacking a LRQLL region and/or a LEMLL region and/or a Zinc Finger motif and/or a glutamine rich domain.

19. A polypeptide comprising all or part of SEQ ID NO:2 fused to a heterologous peptide, preferably to a transcription activating peptide.

20. A nucleic acid molecule encoding a polypeptide of claim 18 or 19.

21. A recombinant expression vector comprising a nucleic acid of claim 20.

22. A recombinant virus encoding a TReP-132 polypeptide comprising all or part of SEQ ID NO:2 or a variant thereof.

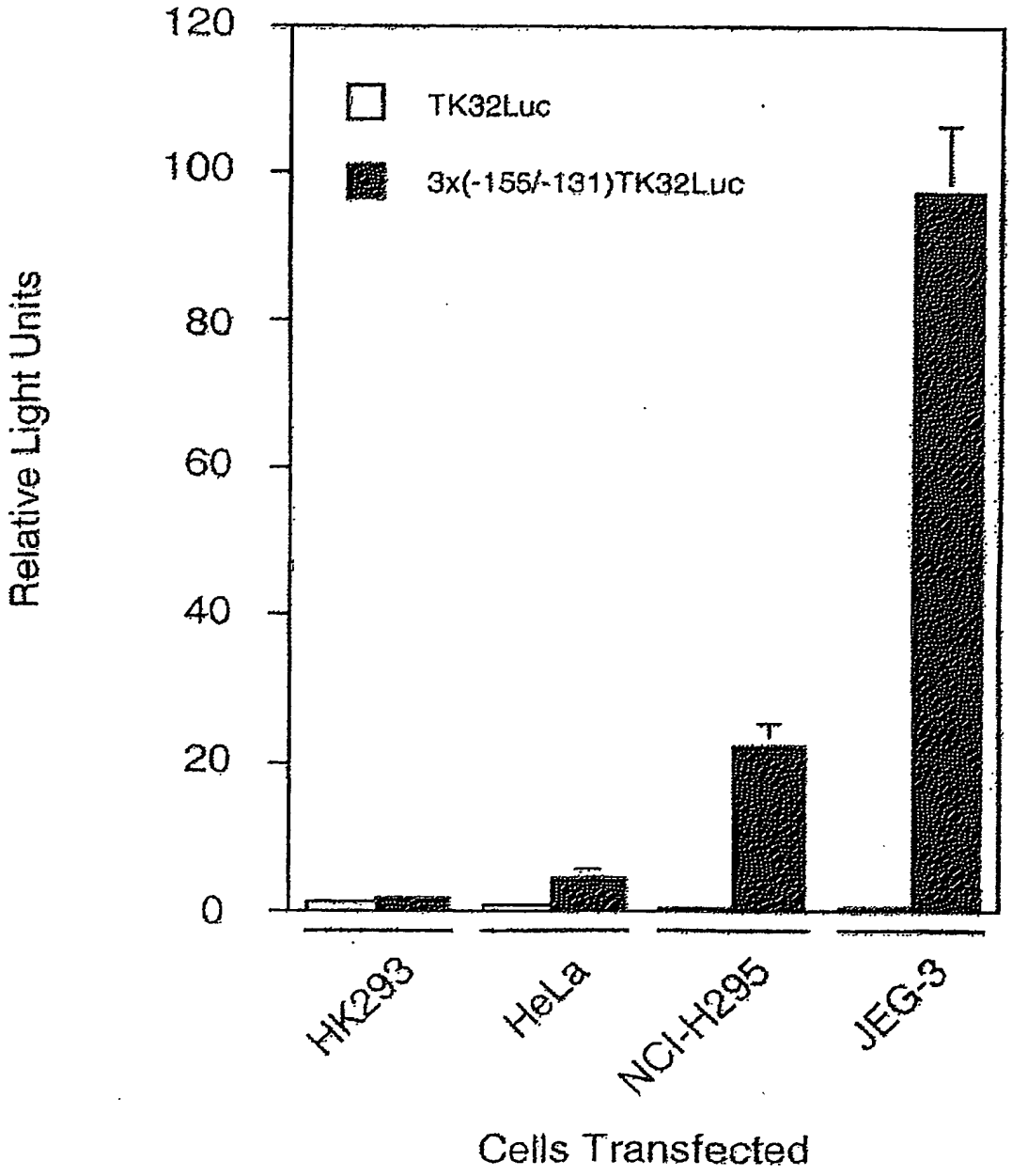
23. A recombinant host cell comprising a nucleic acid molecule of claim 20 or a vector of claim 21 or 22.

24. An antibody that binds a TReP-132 polypeptide comprising all or part of SEQ ID NO:2 or a variant thereof.

25. A pharmaceutical composition comprising a TReP-132 polypeptide or a nucleic acid molecule encoding the same or a compound that modulates expression or activity of a TReP-132 polypeptide.

26. A method of detecting, diagnosing or monitoring the presence of or predisposition to or the evolution of a steroid hormone related disease or a lipid metabolism disorder in a subject, the method comprising determining in vitro the status of the TReP-132 gene or polypeptide in a biological sample from the subject, preferably by detecting the presence of variant(s), polymorphism(s) or genetic alteration(s) in the TReP-132 gene or polypeptide in a biological sample from said subject.

Figure 1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100
80
60
40
20
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3x(-155/-131)TK32Luc
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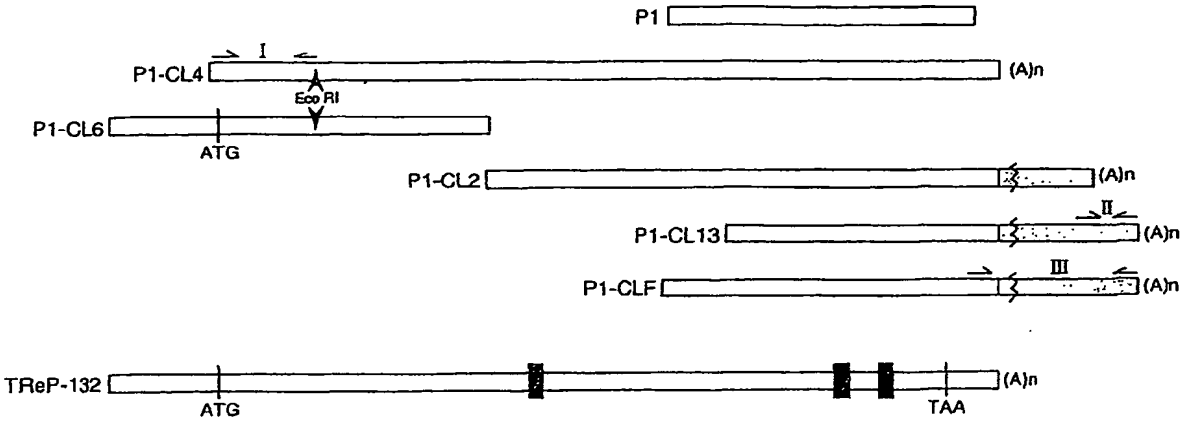
HeLa
NCI-H295
JEG-3
Cells Transfected

Figure 2A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I
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EcoRI
P1-CL6
ATG
P1-CL2
(A)n
II
P1-CL13
(A)n
III
P1-CLF
(A)n
TReP-132
(A)n
ATG
TAA

Figure 2B

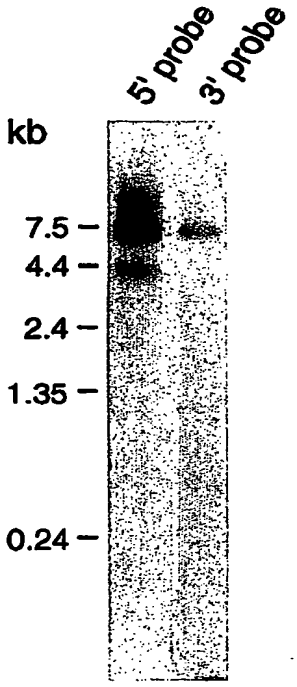


Figure 3A

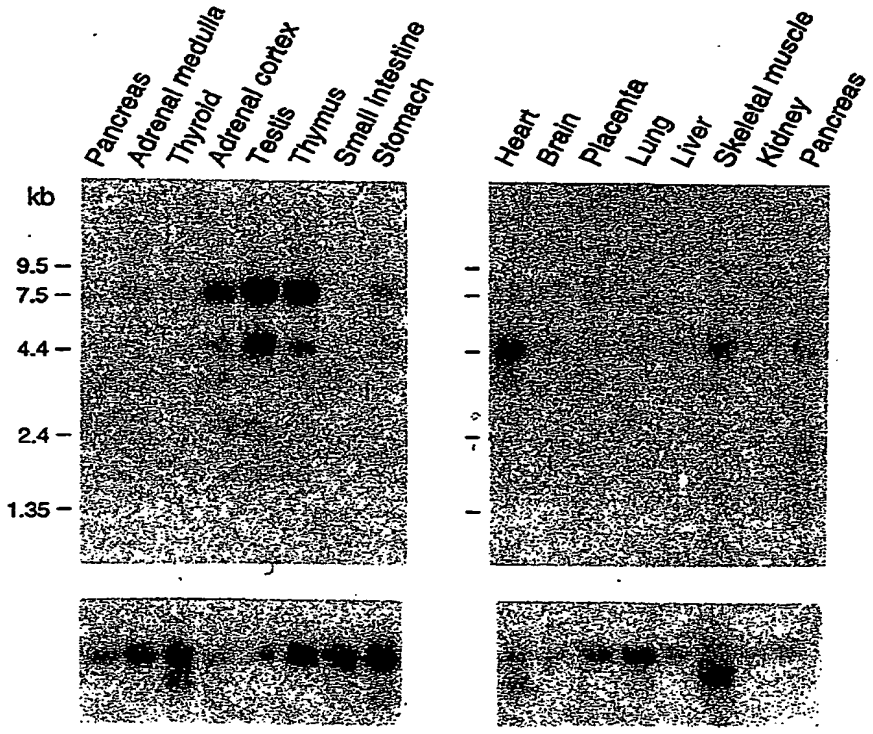
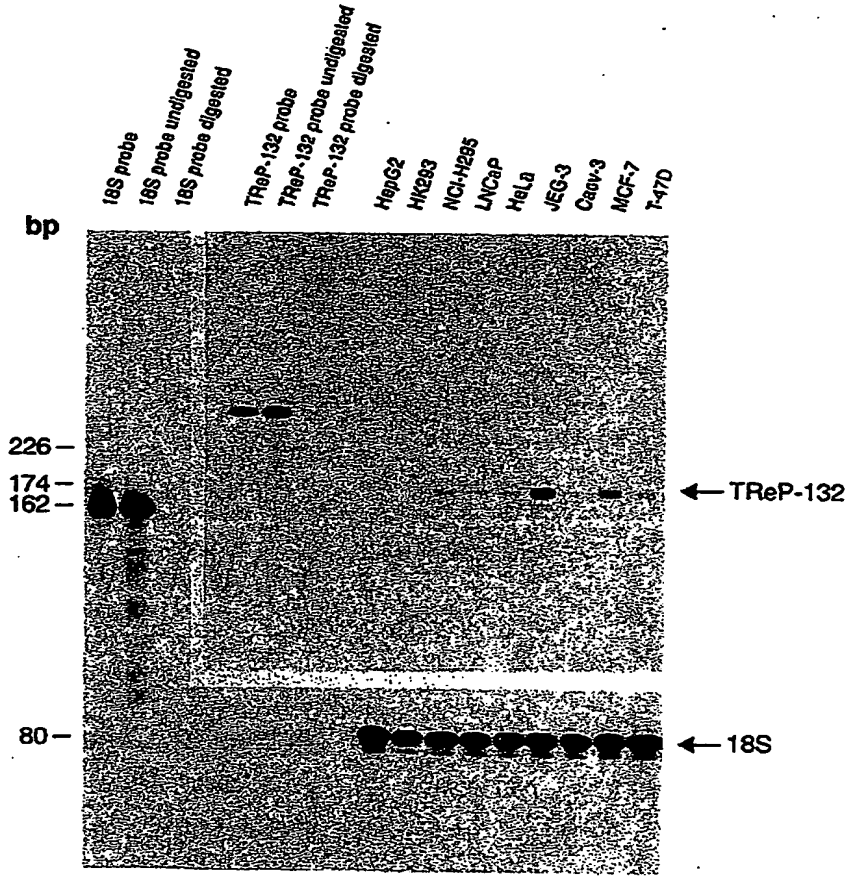

Pancreas
Adrenal medulla
Thyroid
Adrenal cortex
Testis
Thymus
Small Intestine
Stomach
Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas
kb
9.5
7.5
4.4
2.4
1.35

Figure 3B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TReP-132 probe
TReP-132 probe undigested
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LNCaP
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bp
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174
162
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TReP-132
18S

Figure 4

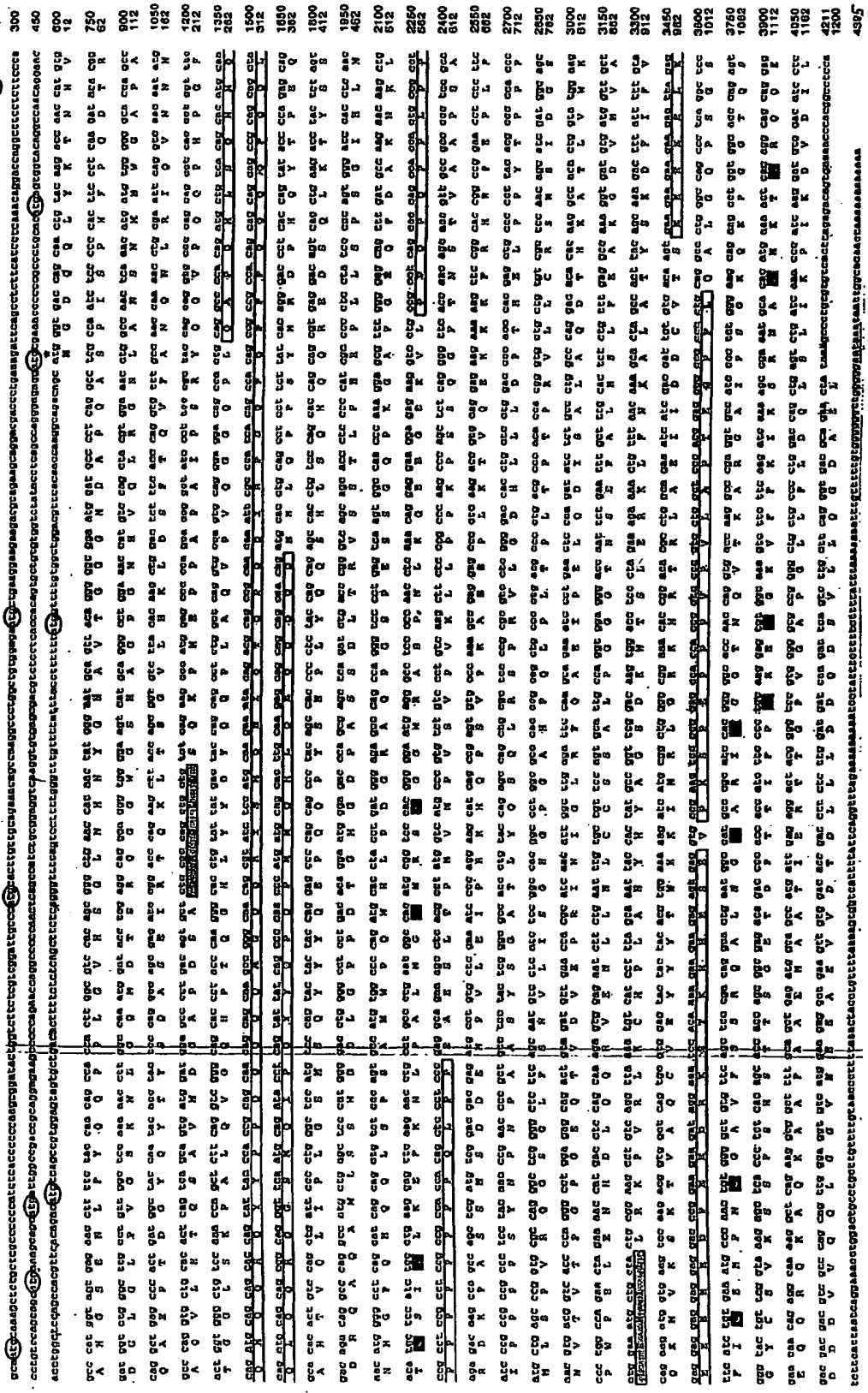


Figure 5A

Fig. 5A

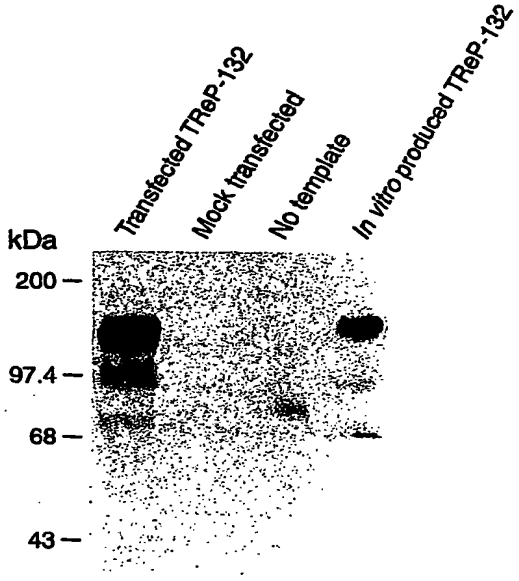


Figure 5B

Fig. 5B

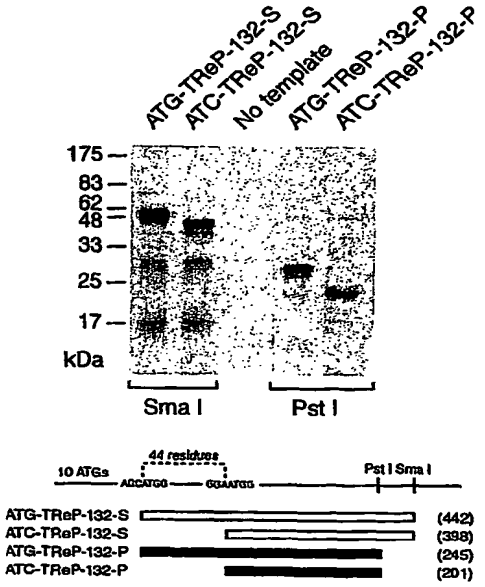


Figure 6

Fig. 6

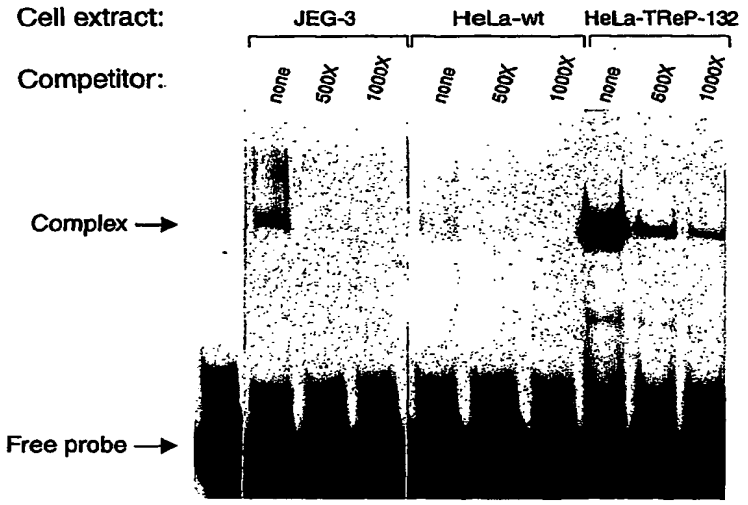


Figure 7

Fig. 7

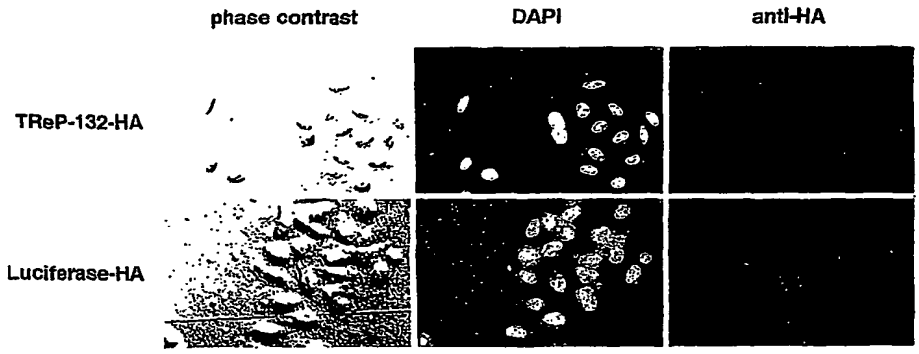
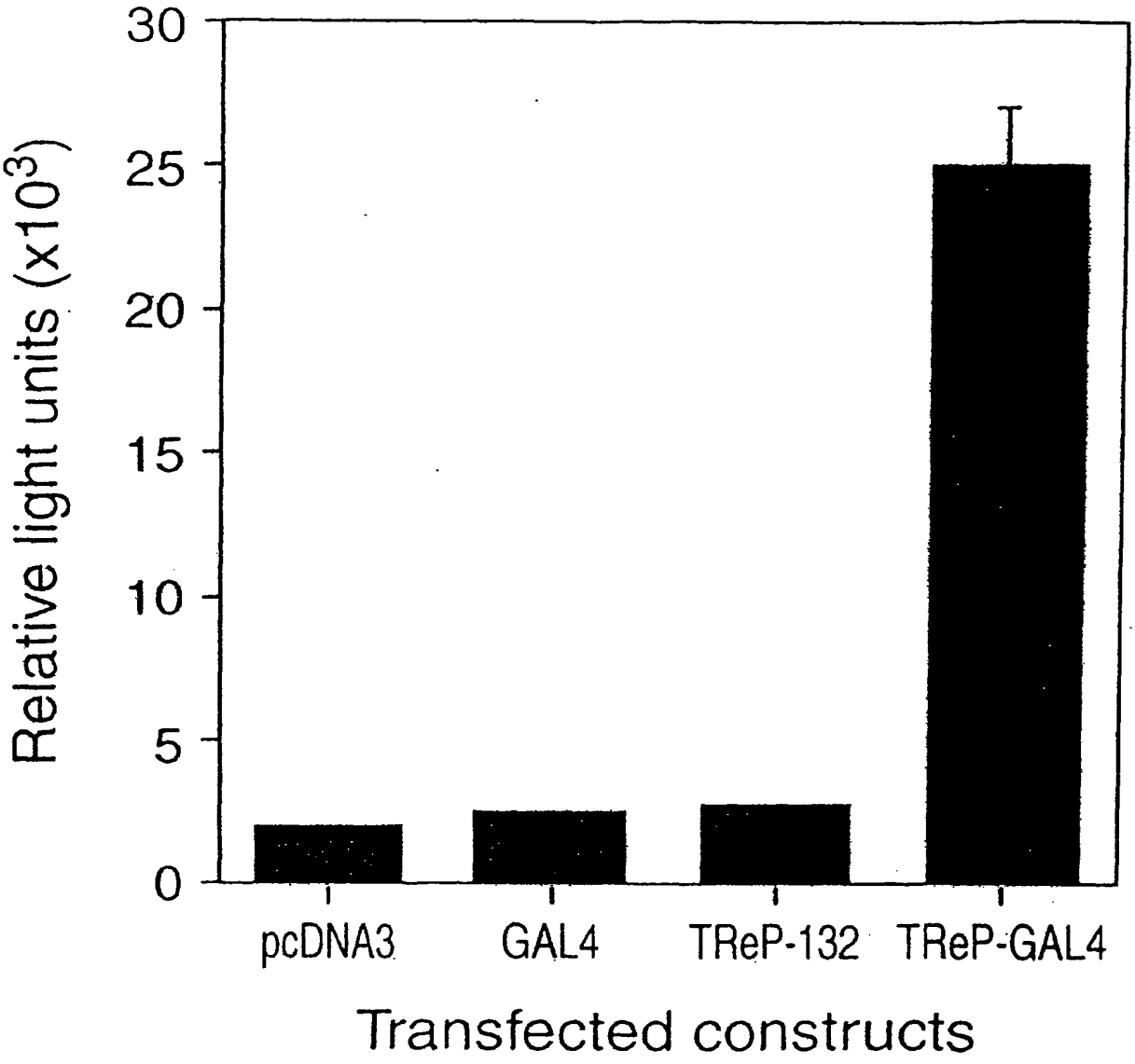


Figure 8A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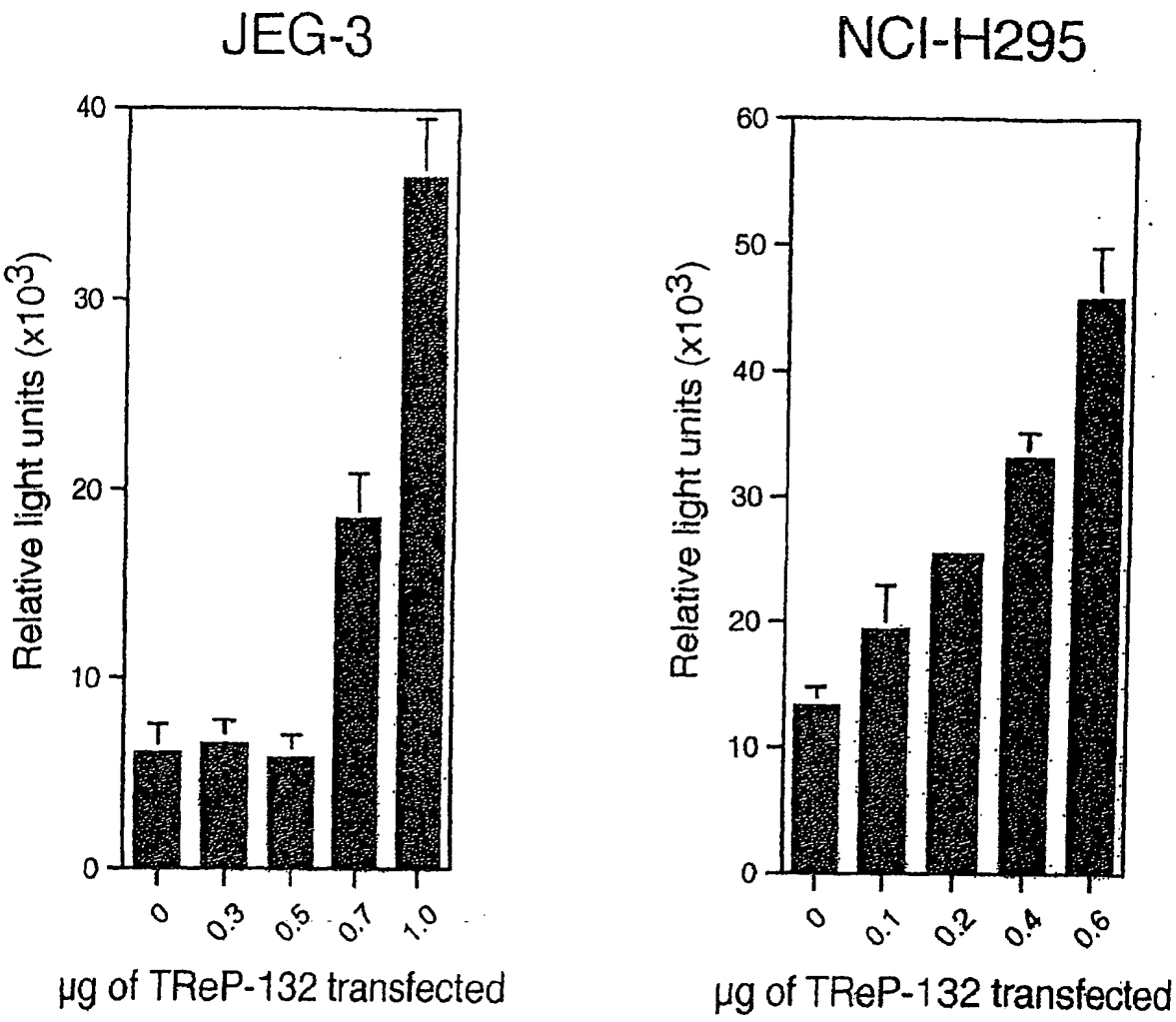
TReP-132
TReP-GAL4
Transfected constructs

Figure 8B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0.5
0.7
1.0
µg of TReP-132 transfected
NCI-H295
Relative light units (x10^3)
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20
10
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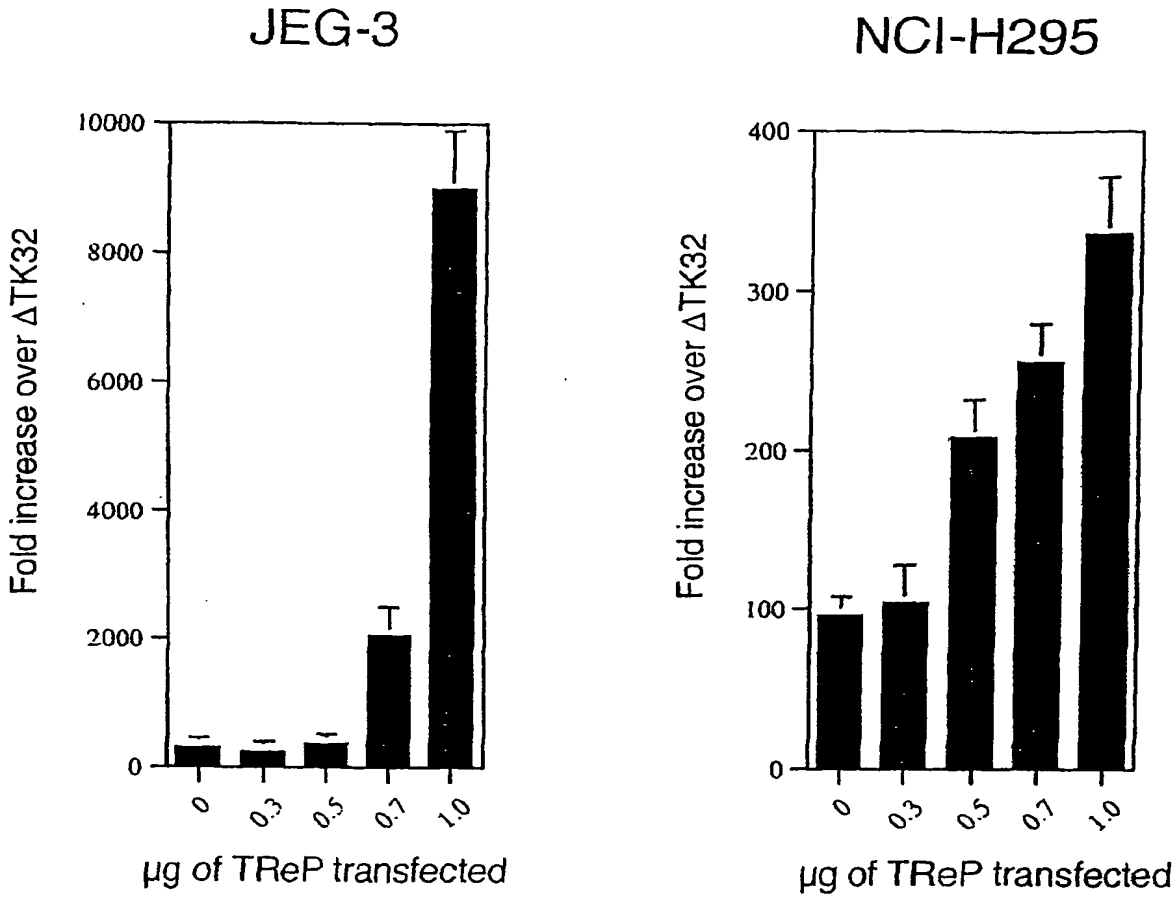
0.2
0.4
0.6
µg of TReP-132 transfected

Figure 8C


JEG-3
Fold increase over ΔTK32
10000
8000
6000
4000
2000
0
0
0.3
0.5
0.7
1.0
µg of TReP transfected
NCI-H295
Fold increase over ΔTK32
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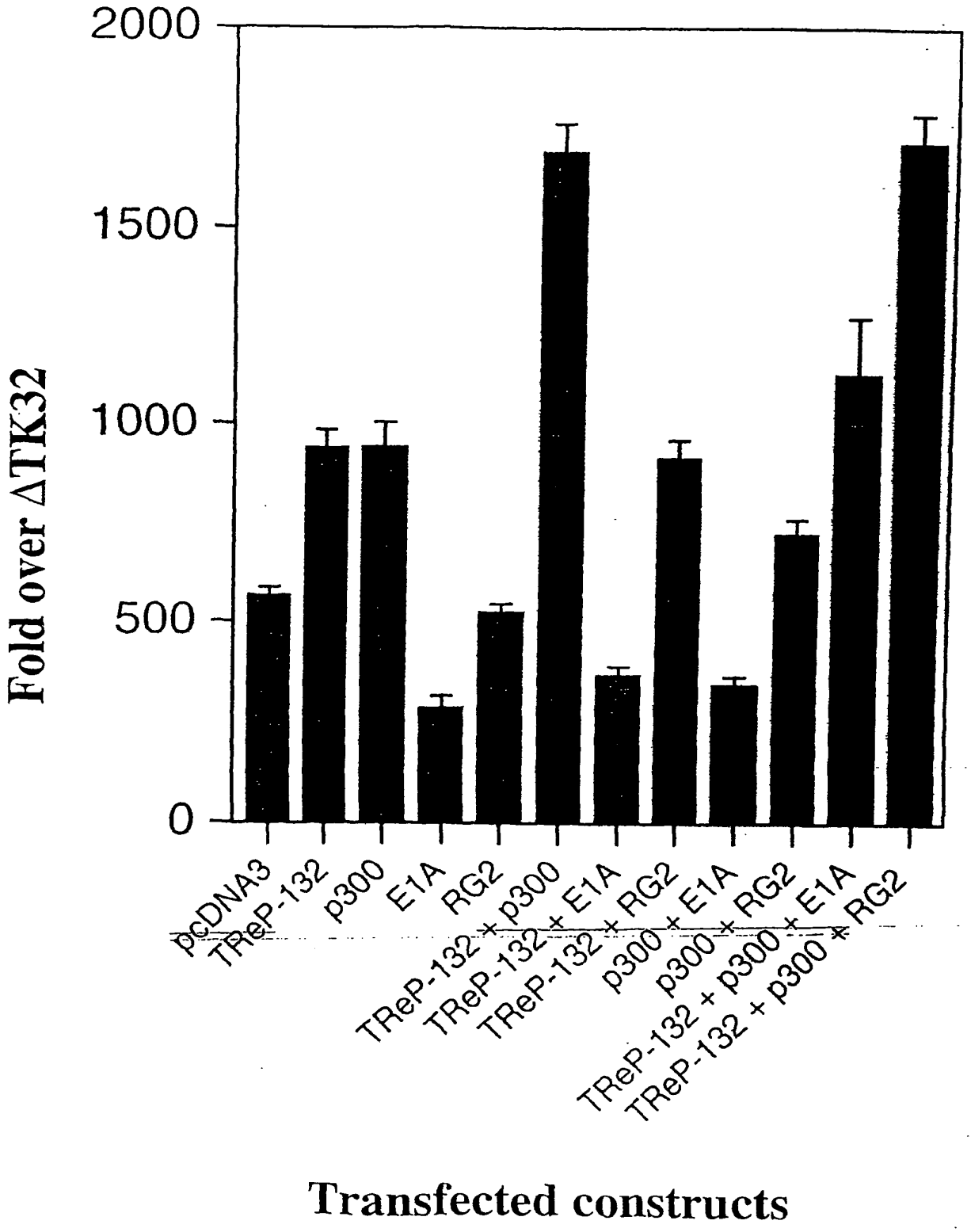
300
200
100
0
0
0.3
0.5
0.7
1.0
µg of TReP transfected

Figure 9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1500
1000
500
0
Fold over ΔTK32
pcDNA3
TReP-132
p300
E1A
RG2
TReP-132 + p300
TReP-132 + E1A
TReP-132 + RG2
p300 + E1A
p300 + RG2
TReP-132 + p300 + E1A
TReP-132 + p300 + RG2
Transfected constructs

Figure 10A (upper pannel)

Fig. 10A (upper panel)

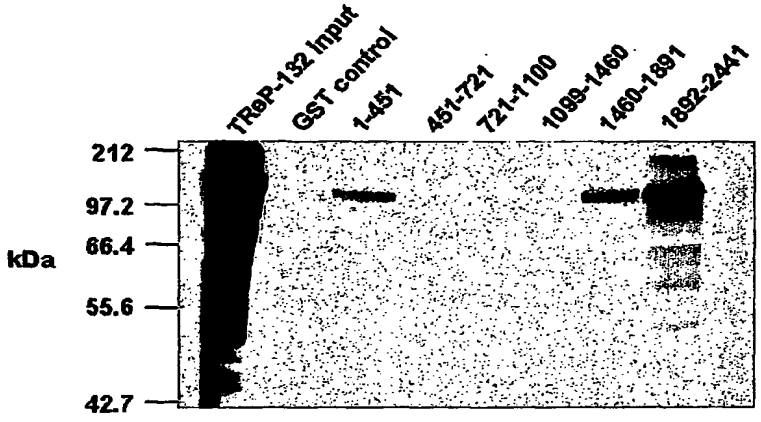


Figure 10A (lower pannel)

Fig. 10A (lower panel)

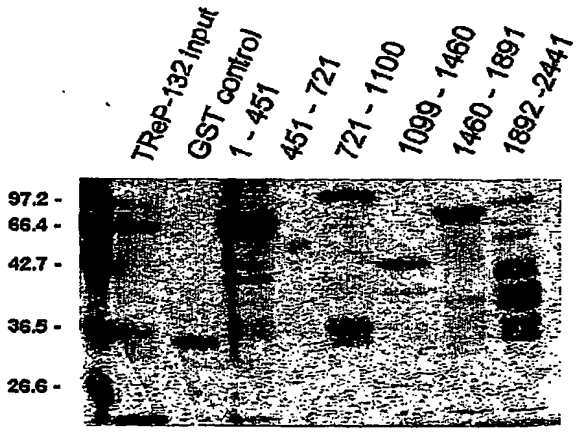
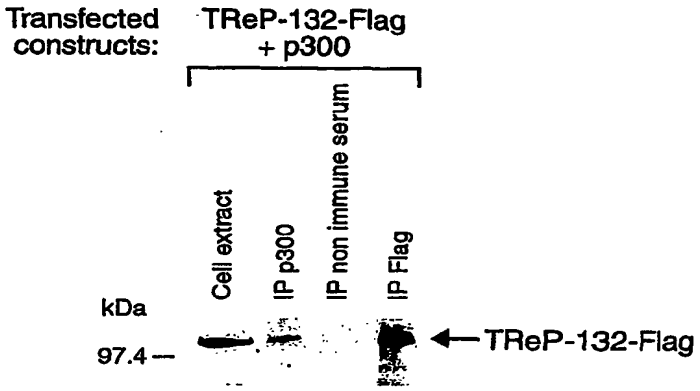
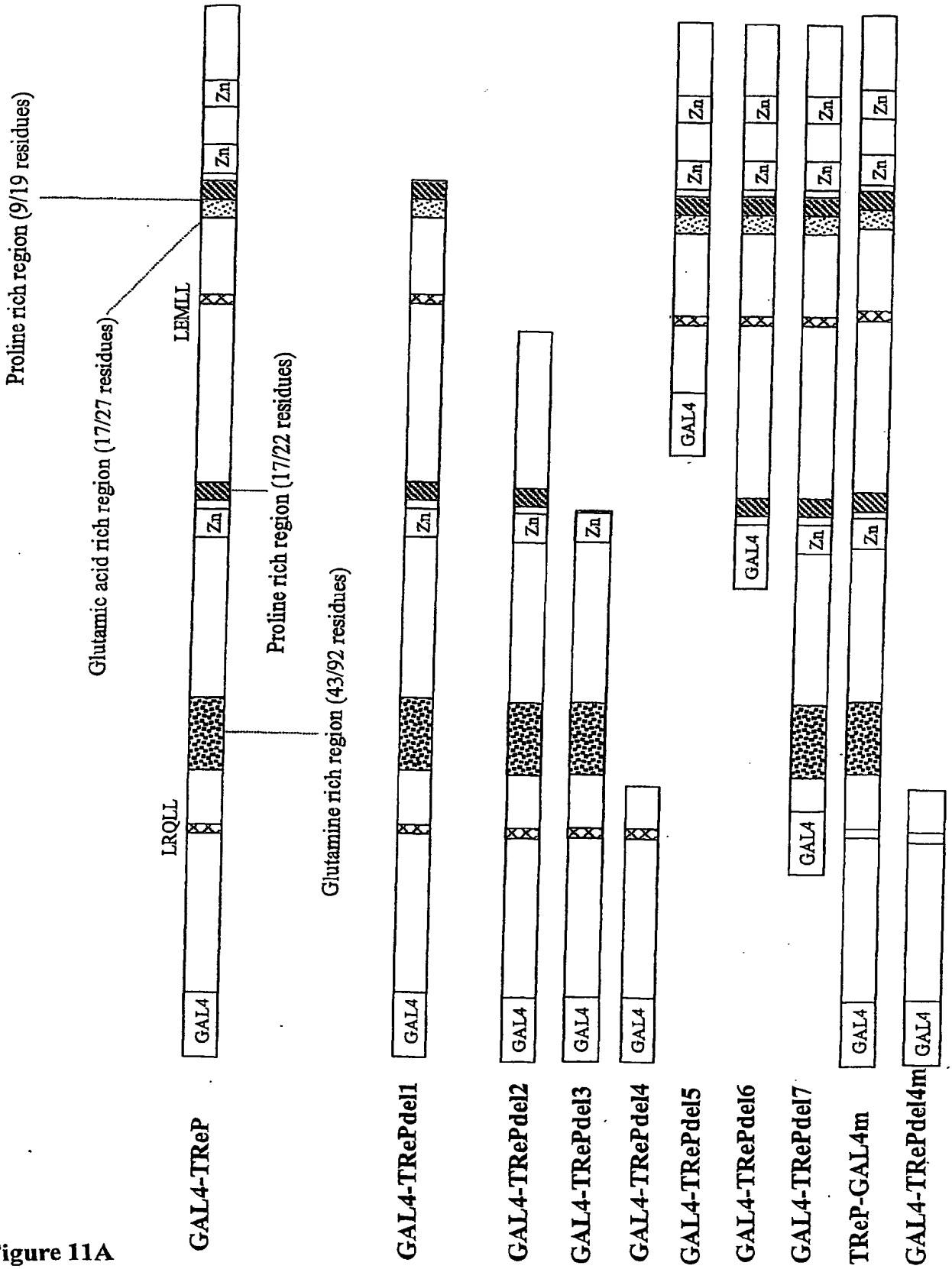


Figure 10B

Fig. 10B

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Glutamic acid rich region (17/27 residues)
LEMLL
Proline rich region (17/22 residues)
Glutamine rich region (43/92 residues)
LRQLL
GAL4
Zn
GAL4-TReP
GAL4-TRePdel1
GAL4-TRePdel2
GAL4-TRePdel3
GAL4-TRePdel4
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GAL4-TRePdel6
GAL4-TRePdel7
TReP-GAL4m
GAL4-TRePdel4m

Figure 11A

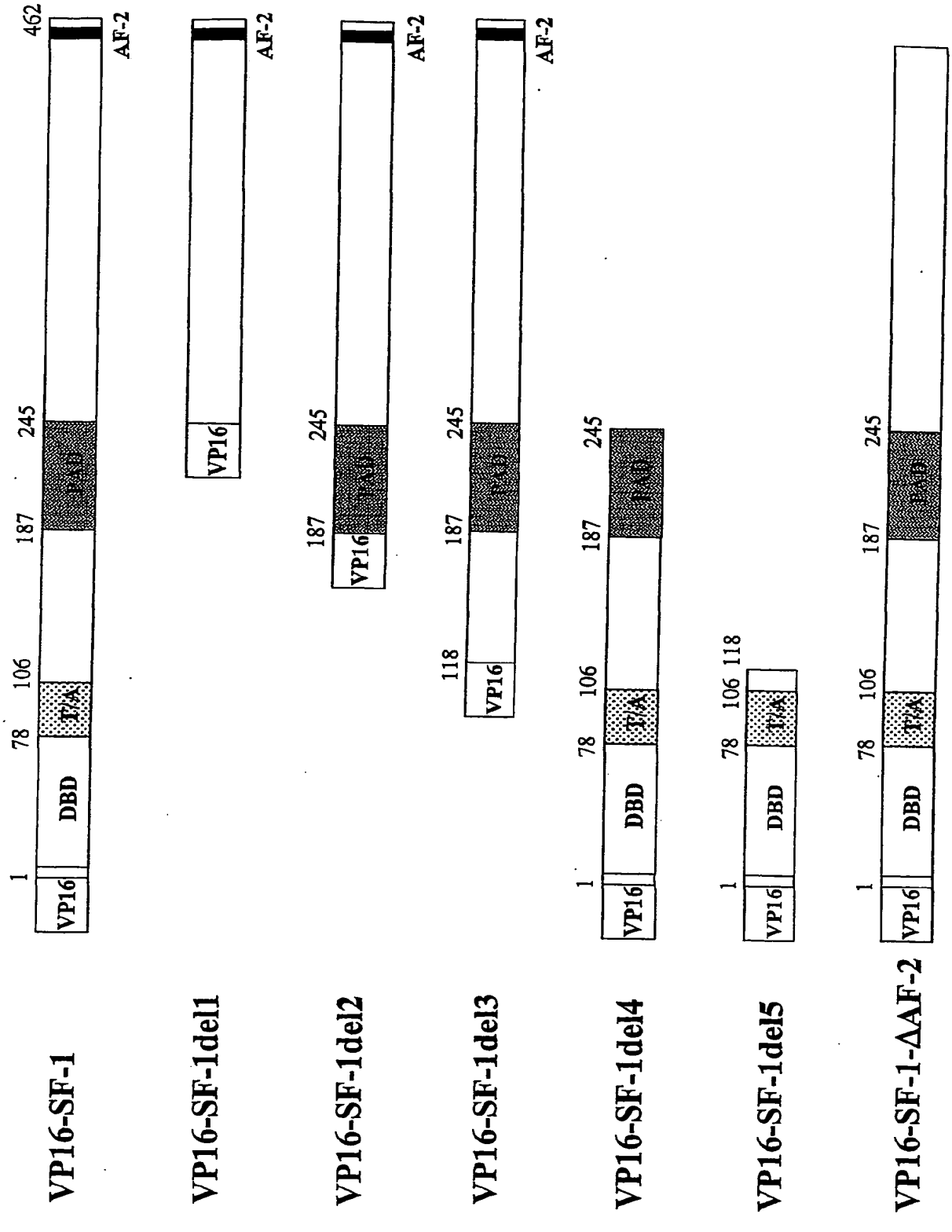
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Figure 11B

SEQUENCE LISTING

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tcatctgtga aatgcccaac tgtggggctg tgttcagctc ccgacaggca ctgaatggcc 3660
atgcccgcat ccacgggggc accaaccagg tgaccaaggc ccgaggtgcc atcccctctg 3720
ggaagcagaa gcctggtggc acccagagtg ggtactgttc ggtaaagagc tcacccctcc 3780
acagcaccac cagcggcgag acagacccca ccaccatctt cccctgcaag gagtgtggca 3840
aagtcttctt caagatcaaa agccgaaatg cacacatgaa aactcacagg cagcaggagg 3900
aacaacagag gcaaaaggct cagaaggcgg cttttgcagc tgagatggca gccacgattg 3960
agaggactac ggggcccgtg ggggcgccgg ggctgctgcc cctggaccag ctgagtctga 4020
tcaaacccat caaggatgtg gacatcctcg acgacgacgt cgtccagcag ttgggaggtg 4080
tcatggaaga ggctgaagtt gtggacaccg atcttctctt ggatgatcaa gattcagtct 4140
tgcttcaggg tgacgcagaa ctataaagcc ctgtgtgtca cttagagaca gtgaaaaccc 4200
acggcctcca tcttcattaa tcaggaaacc tggactgcct gcttgttttg taacccttt 4260

aaactacctg ttttaaaagt ggtcatttta ttcaggttta gaaaaaaaaa tcctatttct 4320
tttcttttat ttaaaaaaat ttgtttttgt ggggggttgg gggaataaat aattggcaca 4380
actaaaaaaa aaaaa 4395

<210> 2
<211> 1200
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primary structure of TRep-132.

<400> 2

Met Gly Asp Gln Gln Leu Tyr Lys Thr Asn His Val Ala His Gly Ser
1 5 10 15

Glu Asn Leu Phe Tyr Gln Gln Pro Pro Leu Gly Val His Ser Gly Leu
20 25 30

Asn His Asn Tyr Gly Asn Ala Val Thr Gly Gly Gly Met Asp Ala Pro
35 40 45

Gln Ala Ser Pro Ile Ser Pro His Phe Pro Gln Asp Thr Arg Asp Gly
50 55 60

Leu Gly Leu Pro Val Gly Ser Lys Asn Leu Gly Gln Met Asp Thr Ser
65 70 75 80

Arg Gln Gly Gly Trp Gly Ser His Ala Gly Pro Gly Asn His Val Gln
85 90 95

Leu Arg Gly Asn Leu Ala Asn Ser Asn Met Met Trp Gly Ala Pro Ala
100 105 110

Gln Ala Glu Pro Thr Asp Gly Tyr Gln Tyr Thr Tyr Ser Gln Ala Ser
115 120 125

Glu Ile Arg Thr Gln Lys Leu Thr Ser Gly Val Leu His Lys Leu Asp
130 135 140

Ser Phe Thr Gln Val Phe Ala Asn Gln Asn Leu Arg Ile Gln Val Asn
145 150 155 160

Asn Met Ala Gln Val Leu His Thr Gln Ser Ala Val Met Asp Gly Ala
165 170 175

Pro Asp Ser Ala Leu Arg Gln Leu Leu Ser Gln Lys Pro Met Glu Pro
180 185 190

Pro Ala Pro Ala Ile Pro Ser Arg Tyr Gln Gln Val Pro Gln Gln Pro
195 200 205

His Pro Gly Phe Thr Gly Gly Leu Ser Lys Pro Ala Leu Gln Val Gly
210 215 220

Gln His Pro Thr Gln Gly His Leu Tyr Tyr Asp Tyr Gln Gln Pro Leu
225 230 235 240

Ala Gln Val Pro Val Gln Gly Gly Gln Pro Leu Gln Ala Pro Gln Met
245 250 255

Leu Ser Gln His Met Gln Gln Met Gln Gln His Gln Tyr Tyr Pro Pro
260 265 270

Gln Gln Gln Gln Gln Ala Gly Gln Gln Arg Ile Ser Met Gln Glu Ile
275 280 285

Gln Thr Gln Pro Gln Gln Ile Arg Pro Ser Gln Pro Gln Pro Pro Pro
290 295 300

Gln Gln Gln Gln Pro Gln Gln Leu Gln Leu Gln Gln Arg Gln Gly Ser
305 310 315 320

Met Gln Ile Pro Gln Tyr Tyr Gln Pro Gln Pro Met Met Gln His Leu
325 330 335

Gln Glu Gln Gln Gln Gln Gln Met His Leu Gln Pro Pro Ser Tyr His
340 345 350

Arg Asp Pro His Gln Tyr Thr Pro Glu Gln Ala His Thr Val Gln Leu
355 360 365

Ile Pro Leu Gly Ser Met Ser Gln Tyr Tyr Tyr Gln Glu Pro Gln Gln
370 375 380

Pro Tyr Ser His Pro Leu Tyr Gln Gln Ser His Leu Ser Gln His Gln
385 390 395 400

Gln Arg Glu Asp Ser Gln Leu Lys Thr Tyr Ser Ser Asp Arg Gln Ala
405 410 415

Gln Ala Met Leu Ser Ser His Gly Asp Leu Gly Pro Pro Asp Thr Gly
420 425 430

Met Gly Asp Pro Ala Ser Ser Asp Leu Thr Arg Val Ser Ser Thr Leu
435 440 445

Pro His Arg Pro Leu Leu Ser Pro Ser Gly Ile His Leu Asn Asn Met
450 455 460

Gly Pro Gln His Gln Gln Leu Ser Pro Ser Ala Met Trp Pro Gln Met
465 470 475 480

His Leu Pro Asp Gly Arg Ala Gln Pro Gly Ser Pro Glu Ser Ser Gly
485 490 495

Gln Pro Lys Gly Ala Phe Gly Glu Gln Phe Asp Ala Lys Asn Lys Leu
500 505 510

Thr Cys Ser Ile Cys Leu Lys Glu Phe Lys Asn Leu Pro Ala Leu Asn
515 520 525

Gly His Met Arg Ser His Gly Gly Met Arg Ala Ser Pro Asn Leu Lys
530 535 540

Gln Glu Glu Gly Glu Lys Val Leu Pro Pro Gln Pro Gln Pro Pro Leu
545 550 555 560

Pro Pro Pro Pro Pro Pro Pro Pro Pro Pro Gln Leu Pro Pro Glu Ala
565 570 575

Glu Ser Leu Thr Pro Met Val Met Pro Val Ser Val Pro Val Lys Leu
580 585 590

Leu Pro Pro Lys Pro Ser Ser Gln Gly Phe Thr Asn Ser Thr Val Ala
595 600 605

Ala Pro Ser Ala Arg Asp Lys Pro Ala Ser Ser Met Ser Asp Asp Glu
610 615 620

Met Pro Val Leu Glu Ile Pro Arg Lys His Gln Pro Ser Val Pro Lys
625 630 635 640

Ala Glu Glu Pro Leu Lys Thr Val Gln Glu Lys Lys Lys Phe Arg His
645 650 655

Arg Pro Glu Pro Leu Phe Ile Pro Pro Pro Pro Ser Tyr Asn Pro Asn
660 665 670

Pro Ala Ala Ser Tyr Ser Gly Ala Thr Leu Tyr Gln Ser Gln Leu Arg
675 680 685

Ser Pro Arg Val Leu Gly Asp His Leu Leu Leu Asp Pro Thr His Glu
690 695 700

Leu Pro Pro Tyr Thr Pro Pro Pro Met Leu Ser Pro Val Arg Gln Gly
705 710 715 720

Ser Gly Leu Phe Ser Asn Val Leu Ile Ser Gly His Gly Pro Gly Ala
725 730 735

His Pro Gln Leu Pro Leu Thr Pro Leu Thr Pro Thr Pro Arg Val Leu
740 745 750

Leu Cys Arg Ser Asn Ser Ile Asp Gly Ser Asn Val Thr Val Thr Pro
755 760 765

Gly Pro Gly Glu Gln Thr Val Asp Val Glu Pro Arg Ile Asn Ile Gly
770 775 780

Leu Arg Phe Gln Ala Glu Ile Pro Glu Leu Gln Asp Ile Ser Ala Leu
785 790 795 800

Ala Gln Asp Thr His Lys Ala Thr Leu Val Trp Lys Pro Trp Pro Glu
805 810 815

Leu Glu Asn His Asp Leu Gln Gln Arg Val Glu Asn Leu Leu Asn Leu
820 825 830

Cys Cys Ser Ser Ala Leu Pro Gly Gly Gly Thr Asn Ser Glu Phe Ala
835 840 845

Leu His Ser Leu Phe Glu Ala Lys Gly Asp Val Met Val Ala Leu Glu
850 855 860

Met Leu Leu Leu Arg Lys Pro Val Arg Leu Lys Cys His Pro Leu Ala
865 870 875 880

Asn Tyr His Tyr Ala Gly Ser Asp Lys Trp Thr Ser Leu Glu Arg Lys
885 890 895

Leu Phe Asn Lys Ala Leu Ala Thr Tyr Ser Lys Asp Phe Ile Phe Val
900 905 910

Gln Lys Met Val Lys Ser Lys Thr Val Ala Gln Cys Val Glu Tyr Tyr
915 920 925

Tyr Thr Trp Lys Lys Ile Met Arg Leu Gly Arg Lys His Arg Thr Arg
930 935 940

Leu Ala Glu Ile Ile Asp Asp Cys Val Thr Ser Glu Glu Glu Glu Glu
945 950 955 960

Leu Glu Glu Glu Glu Glu Glu Asp Pro Glu Glu Asp Arg Lys Ser Thr
965 970 975

Lys Glu Glu Glu Ser Glu Val Pro Lys Ser Pro Glu Pro Pro Pro Val
980 985 990

Pro Val Leu Ala Pro Thr Glu Gly Pro Pro Leu Gln Ala Leu Gly Gln
995 1000 1005

Pro Ser Gly Ser Phe Ile Cys Glu Met Pro Asn Cys Gly Ala Val Phe
1010 1015 1020

Ser Ser Arg Gln Ala Leu Asn Gly His Ala Arg Ile His Gly Gly Thr
1025 1030 1035 1040

Asn Gln Val Thr Lys Ala Arg Gly Ala Ile Pro Ser Gly Lys Gln Lys
1045 1050 1055

Pro Gly Gly Thr Gln Ser Gly Tyr Cys Ser Val Lys Ser Ser Pro Ser
1060 1065 1070

His Ser Thr Thr Ser Gly Glu Thr Asp Pro Thr Thr Ile Phe Pro Cys
1075 1080 1085

Lys Glu Cys Gly Lys Val Phe Phe Lys Ile Lys Ser Arg Asn Ala His
1090 1095 1100

Met Lys Thr His Arg Gln Gln Glu Glu Gln Gln Arg Gln Lys Ala Gln
1105 1110 1115 1120

Lys Ala Ala Phe Ala Ala Glu Met Ala Ala Thr Ile Glu Arg Thr Thr
1125 1130 1135

Gly Pro Val Gly Ala Pro Gly Leu Leu Pro Leu Asp Gln Leu Ser Leu
1140 1145 1150

Ile Lys Pro Ile Lys Asp Val Asp Ile Leu Asp Asp Asp Val Val Gln
1155 1160 1165

Gln Leu Gly Gly Val Met Glu Glu Ala Glu Val Val Asp Thr Asp Leu
1170 1175 1180

Leu Leu Asp Asp Gln Asp Ser Val Leu Leu Gln Gly Asp Ala Glu Leu
1185 1190 1195 1200

<210> 3
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: cis-acting region of the CYPIIA1 gene promoter that is regulated by TReP-132.

<400> 3
gatctcgctg cagaaattcc agactgaacc g 31

<210> 4
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: cis-acting region of the CYPIIA1 gene promoter that is regulated by TReP-132.

<400> 4
gatccggttc agtctggaat ttctgcagcg a 31

<210> 5
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oligonucleotide.

<400> 5
tcaaggcca 9

<210> 6
<211> 463
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Amino acid sequence of SF-1.

<400> 6

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Met Asp Tyr Ser Tyr Asp Glu Asp Leu Asp Glu Leu Cys Pro Val Cys
  1               5                  10                  15

Gly Asp Lys Val Ser Gly Tyr His Tyr Gly Leu Leu Thr Cys Glu Ser
             20                  25                  30

Cys Lys Gly Phe Phe Lys Arg Thr Val Gln Asn Asn Lys His Tyr Thr
         35                  40                  45

Cys Thr Glu Ser Gln Ser Cys Lys Ile Asp Lys Thr Gln Arg Lys Arg
     50                  55                  60

Cys Pro Phe Cys Arg Phe Gln Lys Cys Leu Thr Val Gly Met Arg Leu
 65                  70                  75                  80

Glu Ala Val Arg Ala Asp Arg Met Arg Gly Gly Arg Asn Lys Phe Gly
                 85                  90                  95

Pro Met Tyr Lys Arg Asp Arg Ala Leu Lys Gln Gln Lys Lys Ala Gln
            100                 105                 110

Ile Arg Ala Asn Gly Phe Lys Leu Glu Thr Gly Pro Pro Met Gly Val
        115                 120                 125

Pro Pro Pro Pro Pro Pro Pro Pro Asp Tyr Met Leu Pro Pro Ser Leu
    130                 135                 140

His Ala Pro Glu Pro Lys Ala Leu Val Ser Gly Pro Pro Ser Gly Pro
145                 150                 155                 160

Leu Gly Asp Ile Gly Ala Pro Ser Leu Pro Met Ser Val Pro Gly Pro
                165                 170                 175

His Gly Pro Leu Ala Gly Tyr Leu Tyr Pro Ala Phe Ser Asn Arg Thr
            180                 185                 190

Ile Lys Ser Glu Tyr Pro Glu Pro Tyr Ala Ser Pro Pro Gln Gln Pro
        195                 200                 205

Gly Pro Pro Tyr Ser Tyr Pro Glu Pro Phe Ser Gly Gly Pro Asn Val
    210                 215                 220
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Pro Glu Leu Ile Leu Gln Leu Leu Gln Leu Glu Pro Glu Glu Asp Gln
225 230 235 240

Val Arg Ala Arg Ile Val Gly Cys Leu Gln Glu Pro Ala Lys Ser Gly
245 250 255

Ser Asp Gln Pro Ala Pro Phe Ser Leu Leu Cys Arg Met Ala Asp Gln
260 265 270

Thr Phe Ile Ser Ile Val Asp Trp Ala Arg Arg Cys Met Val Phe Lys
275 280 285

Glu Leu Glu Val Ala Asp Gln Met Thr Leu Leu Gln Asn Cys Trp Ser
290 295 300

Glu Leu Leu Val Leu Asp His Ile Tyr Arg Gln Val Gln Tyr Gly Lys
305 310 315 320

Glu Asp Ser Ile Leu Leu Val Ser Gly Gln Glu Val Glu Leu Ser Thr
325 330 335

Val Ala Val Glu Ala Gly Ser Leu Leu His Ser Leu Val Leu Arg Ala
340 345 350

Gln Glu Leu Val Leu Gln Leu His Ala Leu Gln Leu Asp Arg Gln Glu
355 360 365

Phe Val Cys Leu Lys Phe Leu Ile Leu Phe Ser Leu Asp Val Lys Phe
370 375 380

Leu Asn Asn His Ser Leu Val Lys Asp Ala Gln Glu Lys Ala Asn Ala
385 390 395 400

Ala Leu Leu Asp Tyr Thr Leu Cys His Tyr Pro His Cys Gly Asp Lys
405 410 415

Phe Gln Gln Leu Leu Leu Cys Leu Val Glu Val Arg Ala Leu Ser Met
420 425 430

Gln Ala Lys Glu Gln Tyr Leu Tyr His Lys His Leu Gly Asn Glu Met
435 440 445

Pro Arg Asn Asn Leu Leu Ile Glu Met Leu Gln Ala Lys Gln Thr
450 455 460

<210> 7

<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: TReP-132m1.

<400> 7
Ser Ala Val Met Asp Gly Ala Pro Asp Ser Ala Ala Arg Gln Ala Ala
1 5 10 15

Ser Gln Lys Pro Met
20

<210> 8
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: TReP-132m2.

<400> 8
Leu Phe Glu Ala Lys Gly Asp Val Met Val Ala Ala Glu Met Ala Ala
1 5 10 15

Leu Arg Lys Pro Val
20

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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International Bureau

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PCT

(10) International Publication Number
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(21) International Application Number: PCT/EP02/03893

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(30) Priority Data:
01400923.7 10 April 2001 (10.04.2001) EP

(71) Applicant *(for all designated States except US)*: **GENFIT** [FR/FR]; Parc Eurasanté, Lille Métropole, 885, Avenue Eugène Avinée, F-59120 Loos (FR).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **HUM, Dean, W.** [CA/FR]; 26, Allée des Fenaisons, F-59910 Bondues (FR).

(74) Agents: **BECKER, Philippe** et al.; Cabinet Becker et Associés, 35, rue des Mathurins, F-75008 Paris (FR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
23 January 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/083726 A3

(54) Title: A NOVEL ZINC FINGER PROTEIN AND USES THEREOF

(57) Abstract: The present invention relates to the identification of a novel protein, TReP-132, involved in the regulation of gene expression. This invention relates to the characterization of the function of this protein, particularly as a transcription factor involved in steroidogenesis, as well as of metabolic partners thereof. This invention also includes compositions and methods of using said protein or corresponding nucleic acids, as well as variants thereof, for screening, diagnostic and/or therapeutic purposes, more particularly for diagnosis of, therapy of, or screening compounds active in steroid hormone related disorders or in lipid disorders.

INTERNA[redacted]L SEARCH REPORT

Int[redacted]pplication No
PC[redacted]2/03893

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/47 G01N33/50 C12Q1/68 C12N15/86
C07K16/18 A61K48/00 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, SEQUENCE SEARCH, BIOSIS, MEDLINE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUANG N ET AL: "CLONING OF FACTORS RELATED TO HIV-INDUCIBLE LBP PROTEINS THAT REGULATE STEROIDOGENIC FACTOR-1-INDEPENDENT HUMAN PLACENTAL TRANSCRIPTION OF THE CHOLESTEROL SIDE-CHAIN CLEAVAGE ENZYME, P450SCC" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 275, no. 4, 28 January 2000 (2000-01-28), pages 2852-2858, XP002946721 ISSN: 0021-9258 page 2853 -page 2857 -/--	11,16,17

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2002	25/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fotaki, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte[redacted]plication No
PCT[redacted]/03893

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUM D W ET AL: "CHARACTERIZATION OF PLACENTAL TRANSCRIPTIONAL ACTIVATION OF THE HUMAN GENE FOR P450SCC" DNA, MARY ANN LIEBERT, NEW YORK, NY, US, vol. 5, no. 14, 1995, pages 451-463, XP001056580 ISSN: 1044-5498 page 453-456	11,16,17
X	MONTE D ET AL: "REGULATION OF THE HUMAN P450SCC GENE BY STEROIDOGENIC FACTOR 1 IS MEDIATED BY CBP/P300" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 8, no. 273, 20 February 1998 (1998-02-20), pages 4585-4591, XP001056582 ISSN: 0021-9258 page 4589 -page 4591	12-17
X	LIU Z ET AL: "MOLECULAR MECHANISM FOR COOPERATION BETWEEN SP1 AND STEROIDOGENIC FACTOR-1 (SF-1) TO REGULATE BOVINE CYP11A GENE EXPRESSION" MOLECULAR AND CELLULAR ENDOCRINOLOGY, AMSTERDAM, NL, vol. 1/2, no. 153, 1999, pages 183-196, XP001062828 ISSN: 0303-7207 page 183-196	12-17
X	DATABASE EMBL 'Online! retrieved from EBI /EMBL Database accession no. AJ277275 XP002193753 the whole document	18-24
X	DATABASE EMBL 'Online! retrieved from EBI / EMBL Database accession no. Q9NQ72 XP002193754 the whole document	18-24
T	GIZARD FLORENCE ET AL: "A novel zinc finger protein TReP-132 interacts with CBP/p300 to regulate human CYP11A1 gene expression" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 276, no. 36, 7 September 2001 (2001-09-07), pages 33881-33892, XP002193752 ISSN: 0021-9258	

INTERNATIONAL SEARCH REPORT

application No.
/EP 02/03893

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 2-6 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 3-6, 25
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 3-6, 25

Present claims 3-6 (entirely), 25 (partially) relate to an extremely large number of possibl ecompounds. These compounds are described solely in functional terms as "compound that modulates expression or activity of a TReP-132 polypeptide". In view of the lack of any technical features characterizing said compounds, support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is lacking. Therefore, a meaningful search is impossible. Search has been carried out for those parts of claim 25 that relate to a TReP-132 polypeptide or nucleic acid encoding the same.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.